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Reflex Control of Respiration in the Anesthetized Dog during Prolonged Exposure to Positive Radial Acceleration

By

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Abstract

Barr, P.-O., H. Bjurstedt and J. C. G. Coleridge. — Reflex control of respiration in the anesthetized dog during prolonged exposure to positive radial acceleration. *Acta physiol. scand.* 1959. *47.* 1—15. — The ventilatory response of anesthetized dogs to accelerative forces in the head-to-tail direction was investigated using a large centrifuge. An initial apnea was observed, which could be prevented by abdominal counterpressure or bilateral vagotomy. Hyperventilation sometimes followed the apnea and predominated when the vagi had been cut prior to the exposure. Evidence was obtained for a pulmonary stretch reflex origin of the apnea, and for a predominantly chemoreflex drive, resulting from reduced flow through the carotid bodies, when hyperventilation occurred. Thus, reflexogenic control of respiration was prepotent during gravitational stress, whether manifested by inhibition or stimulation of respiration.

Information is needed as to whether deterioration in the performance of pilots subjected to long-lasting accelerative forces may be mediated through disturbances in the normal gas exchange of the lungs. Exploratory experiments

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were therefore performed on anesthetized dogs in this laboratory using a large centrifuge.

In the present paper an account will be given of the changes in pulmonary ventilation observed in anesthetized dogs during prolonged exposure to positive radial acceleration; evidence will be presented that the sometimes impressive respiratory changes were mediated in part through pulmonary stretch reflexes and in part also through intense stimulation of peripheral chemoreceptors. A preliminary account of these findings has appeared (BARR, BJURSTEDT and COLERIDGE 1958).

The associated changes in arterial O_2 saturation and pH will be treated separately in a subsequent paper (BARR, BJURSTEDT and COLERIDGE 1959); this seems justified since they could not be explained solely on the basis of alterations in pulmonary ventilation, but were to a great extent dependent on changes in the pulmonary circulation brought about by the accelerative force.

Methods

Experiments were performed on dogs anesthetized by either 1) chloralose (0.1 g/kg i.v.); 2) morphine sulphate (1 mg/kg s.c.) and chloralose (0.1 g/kg i.v.); or 3) morphine sulphate (3 mg/kg s.c.) and 0.25 ml/kg of a 1 : 1 mixture of Dial-urethane (allobarbitone-urethane, Ciba) and sodium pentobarbital solutions i.v.

For producing prolonged accelerative forces a human centrifuge (24 foot radius) was used, with the animal on a platform at one end of the double-arm superstructure. The animal was placed in the supine position on a V-shaped support, with the long axis of the body in the radius and the head towards the center of rotation. The animal, recording instruments, bags containing different gas mixtures etc. were enclosed in a cabin to avoid interference from winds set up by the rotation of the centrifuge superstructure. To prevent the animal from being displaced in the centrifugal direction during runs, its buttocks were firmly supported by a "seat", moulded to the contours of the body, while the head and limbs were firmly secured to a supporting frame.

Recordings. The general experimental arrangement is shown in Fig. 1. Respiratory movements of the thorax, and sometimes of the abdomen, were recorded with a stethograph connected to a Statham strain-gage manometer (type P23B). The respiratory minute volume was measured by means of a low-resistance gas meter on the inspiratory side of a respiratory valve connected to a tracheal cannula. The inspired gas volumes were recorded by means of a Helipot ten-turn spiral potentiometer attached to the shaft of the gas meter; by using a Wheatstone bridge arrangement a current was obtained that increased linearly with the rotation of the shaft.

To maintain the flow in the carotid arteries as intact as possible during centrifugation, arterial pressures were not recorded directly from these arteries. However, it was felt that information was needed on the pressure changes occurring at the level of the carotid sinus. For this purpose the pressure in the left femoral artery was recorded via a rigid plastic tube connected to a Statham strain-gage manometer (type P23D). The pressure was then translated into that obtaining in the carotid sinus region by placing the strain-gage so that the sinus region and the strain-gage were equidistant from the center of rotation.

The pH of the arterial blood was recorded continuously with a glass electrode assembly

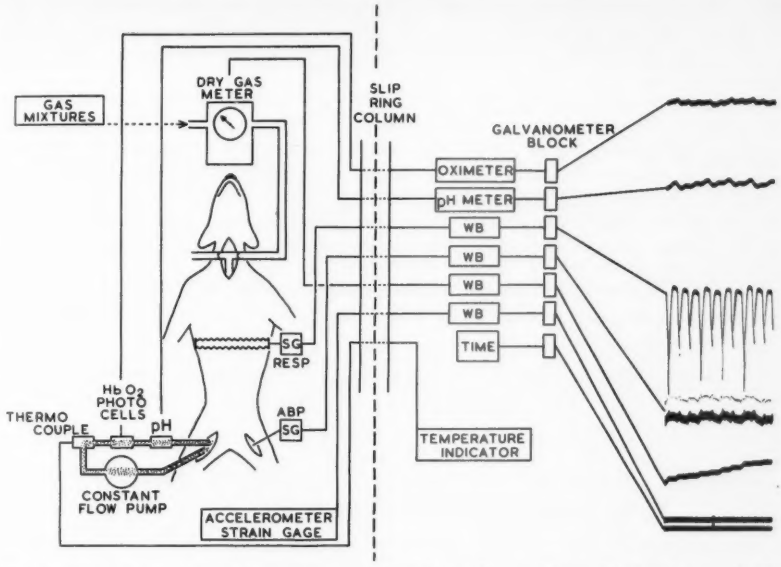

Fig. 1. General experimental arrangement. Dog in supine position with head towards the center of rotation and long axis of body parallel to radius of rotation. Tracheal cannula fitted with inspiratory and expiratory valves; dog inspired through gas meter. Stippling represents arterial blood passing from right femoral artery through loop for the recording of pH and oxygen saturation. Arterial blood pressure (ABP) recorded from left femoral artery with strain-gage (SG) (in experiment placed at the level of the carotid sinus). Thoracic respiration (Resp) was recorded with a stethograph and strain-gage (SG). The various devices connected via a slip-ring column to oximeter, pH meter and Wheatstone bridge arrangements (WB) which in turn were coupled to galvanometers for photographic recording (extreme right).

(BJURSTEDT 1946) connected to the central end of the right femoral artery. A photocell unit for oximetry could also be included, to be described more fully in a subsequent paper (BARR, BJURSTEDT and COLERIDGE 1959). To avoid errors in the oximeter recordings because of changes in flow, or in the pH recording due to changes in the temperature of the blood in the assembly secondary to changes in flow, the blood was directed from the femoral artery to the femoral vein on the same side via a roller pump, which gave a constant flow of 2 ml/sec independently of the artery-vein pressure gradient (Fig. 1). Coagulation was prevented by 1 ml of a 5 % solution of heparin per 10 kg body weight intravenously.

All currents and voltages from measuring devices were led via a central slip-ring arrangement and recorded as shown in Fig. 1.

Dissection. The vagi and the carotid sinus regions on both sides were exposed, and loose ligatures were placed around the vagi and also around all nervous structures originating from the carotid body and sinus regions on both sides. Complete denervation of the carotid bodies and sinus regions could then be accomplished when desired by tying the ligatures, care being taken to avoid interference with the blood flow through

the external and internal carotid arteries. Denervation of the carotid bodies was not considered complete unless inhalation of low O_2 mixtures failed to produce stimulation of respiration (the aortic chemoceptor pathways having previously been interrupted by bilateral vagotomy).

Definition of g and description of runs. In all centrifuge runs the animals were subjected to a resultant force, the direction and magnitude of which at any given instant was the vector sum of gravitational, fictitious centrifugal and tangential forces. In accordance with the convention used in aviation medicine, accelerative forces acting in the head-to-tail direction are always positive (so-called "positive acceleration"). The degree of "positive radial acceleration" to which the animals were exposed therefore corresponded to the centrifugal vector, which is here expressed in *g*-units (for theoretical treatment, see DIXON and PATTERSON 1953).

The centrifugal force was usually applied to reach a maximum within 5 to 10 sec; the force was then maintained for several minutes, thus appearing as a plateau of varying duration in the accelerogram (Fig. 2—6).

Results

General. A total of 154 runs, lasting from 0.5 to 5 min, were made on 14 dogs.

When the centrifugal force exceeded + 3 *g*, cardiovascular and/or respiratory failure frequently developed even after brief exposures. Since we were interested in the effects produced by exposures of several minutes' duration, the results to be described refer to experiments in which centrifugal forces less than + 3 *g* were applied.

The alterations in the systemic arterial pressure and heart rate observed in the present experiments were similar to those reported previously by other investigators (for reviews see WOOD *et al.* 1946 and GAUER 1950); hence, they will be reported only briefly here. A typical record from a prolonged run at a low *g* level is shown in Fig. 2 A. At the onset of centrifugation the arterial blood pressure at the level of the carotid sinus fell, and the heart rate increased; several seconds later a secondary recovery in the arterial pressure occurred, which was never complete at carotid sinus level. The recovery in arterial pressure required intact vagal and carotid sinus nerves; for it was diminished by vagotomy and abolished by additional ligation of the sinus nerves. The arterial pressure at any other level could be calculated from the weight of the blood, which increased in direct proportion to the number of *g* units applied, and from the distance (with respect to the direction of the *g* force) to the carotid sinus level. For example in the experiment shown in Fig. 2 A the mean arterial pressure before the run was approximately 105 mm Hg (at both carotid sinus and femoral artery levels). The animal was then exposed to a centrifugal force of 1.7 *g*. At the lowest point of the initial fall the mean arterial pressure at the carotid sinus level was approximately 20 mm Hg; hence 650 mm caudal to this region (*i. e.* at the femoral artery level) the mean arterial pressure was

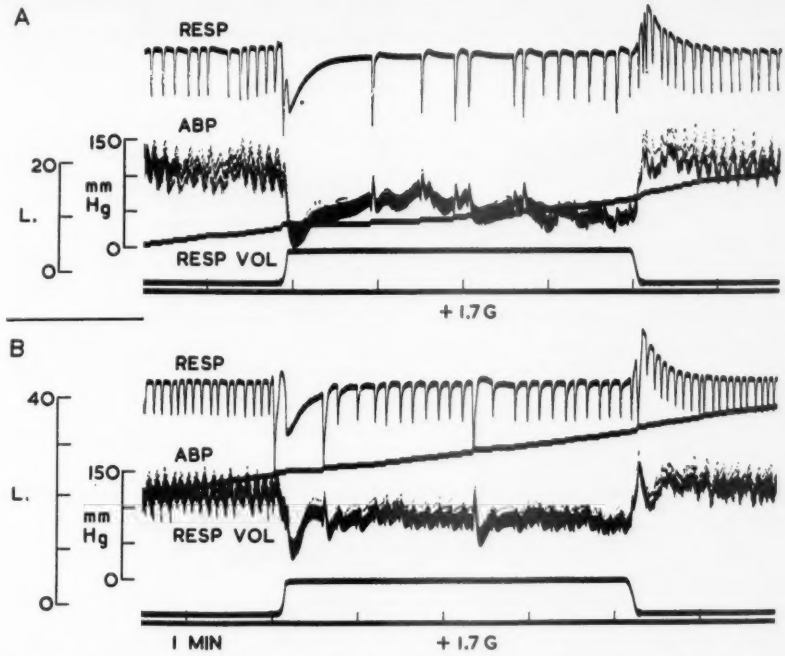

Fig. 2. Effects of prolonged positive acceleration. Dog 20 kg; morphine sulphate, Dial-urethane and sodium pentobarbital; breathing air. Vagus and carotid sinus nerves intact.

A, no external counterpressure applied to the abdomen;

B, cuff inflated to support abdomen.

In these and Fig. 3, 4 and 6 from above downwards: thoracic respiratory movements (Resp) inspiration downwards; arterial blood pressure (ABP) at carotid sinus level; gas meter tracing (Resp vol) slope of which increases with the respiratory minute volume (calibration at the extreme left, in liters; each step in the tracing represents an inspiration); accelerogram starting at and returning to zero *g* in the head-to-tail direction, plateau showing duration and magnitude of the positive acceleration as centrifuge is rotating. Time: 1 min between markings.

(650 × 1.7/13.6) 81 mm Hg higher or a total of approximately 101 mm Hg. In other words the pressure at the level of the femoral artery at this moment was approximately the same as immediately before the onset of centrifugation.

Respiratory responses. The pattern of respiratory response varied with the magnitude as well as with the duration of the centrifugal force. However, when the dog had its vagi intact and did not have its abdominal wall supported by an inflated cuff (see below), apnea invariably occurred at the onset of centrifugation. This initial apnea is well illustrated by a typical record shown in Fig. 2 A (breathing air) and by Fig. 3 A and B (breathing O_2). The apnea

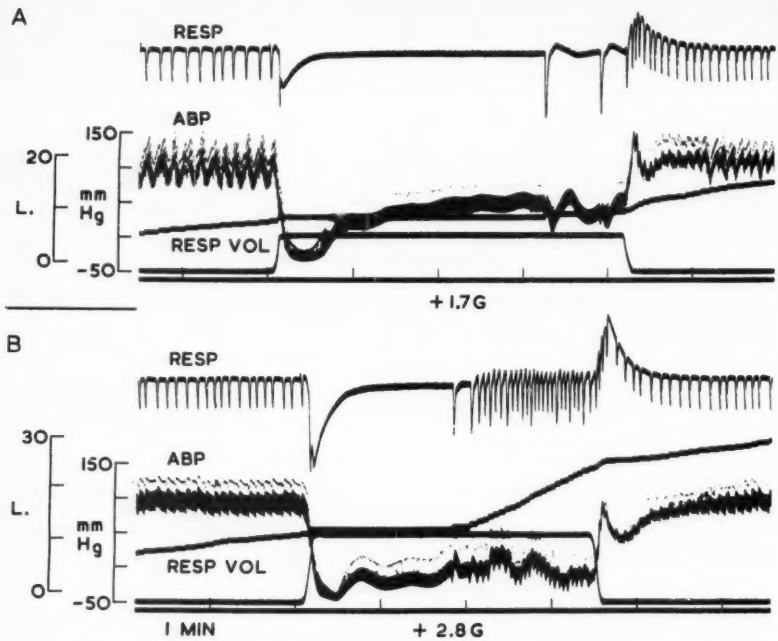

Fig. 3. Effects of prolonged positive acceleration. Same dog as in Fig. 2; breathing 100 % oxygen, no external counterpressure to abdomen. Vagus and carotid sinus nerves intact. A, 1.7 *g*; B, 2.8 *g*. Recordings as in Fig. 2.

was sometimes preceded by a single inspiration, shown clearly by the stethograph tracing. By having the dog breathe 100 % O_2 for a few minutes prior to and during the run, the duration of the initial apnea markedly increased, in some cases lasting for several minutes (Fig. 3 A). The initial apnea differed from respiratory failure in that animals resumed breathing before the runs were completed (see below).

While for a given centrifugal force the duration of the initial apnea could always be increased by O_2 inhalation, a higher *g* level tended to cut short the apnea. This is illustrated by comparison of the records shown in Fig. 3 A and B. At 1.7 *g* the apnea lasted for 185 sec in this dog when breathing O_2; when exposed to 2.8 *g* the animal remained apneic for 95 sec.

During exposure to relatively low *g* levels the initial apnea usually was followed by a slowly increasing respiratory activity, both rate and minute volume at first being greatly below normal. A secondary hyperventilation after the initial apnea could, however, be induced in the majority of dogs

by prolonging the exposure to a given level of *g* or by applying a greater centrifugal force. An abrupt change from apnea to marked hyperventilation could sometimes be observed at higher *g* levels, as shown in Fig. 3 B.

Effect of abdominal support. In several of the present experiments a qualitative record of the abdominal circumference was made with a stethograph coupled to a strain-gage. It was found that the abdominal circumference always increased during positive acceleration, thus pointing to a lowering of the diaphragm and a stretching of the lung tissue in the head-to-tail direction. To determine whether this had any effect upon the pattern of ventilation a sphygmomanometer cuff (12 cm wide) was placed around the upper abdomen and inflated to restrict the outward movement of the abdominal wall and thus to limit the descent of the diaphragm during centrifugation. This maneuver always curtailed or even abolished the initial period of apnea. For example, in the experiment depicted in Fig. 2 A with the dog breathing air and with the deflated cuff in position around the abdomen, positive acceleration caused an apnea lasting 60 sec. The cuff was then inflated to support the abdomen; centrifugation now caused an apnea of only 25 sec (Fig. 2 B). In addition, ventilation in the post-apneic period was more vigorous than in the previous experiment (Fig. 2 A); the respiratory minute volume towards the end of the run was only slightly less than before centrifugation.

The application of counterpressure to the abdomen thus tended to improve ventilation during the runs. Apparently it also reduced the amount of abdominal venous pooling during centrifugation, since for any given level of *g* the fall in arterial pressure was smaller.

Effects of vagotomy. To determine whether the lowering of the diaphragm and the stretching of the lungs in the head-to-tail direction, described above, might reflexly affect respiration by stimulation of pulmonary stretch receptors, the effects of bilateral cervical vagotomy were studied.

As a result of such vagotomy the respiratory response to centrifugation was radically altered. The initial apnea no longer occurred; furthermore, in all but a few cases to be discussed more fully below, the vagotomized animal showed marked hyperventilation during centrifugation. This is illustrated by a comparison of the records shown in Fig. 2 A and 4 in which the animal was subjected to 1.7 *g* before and after vagotomy, respectively. With the vagi intact, ventilation was less during centrifugation than during the pre-run period (Fig. 2 A); whereas after vagotomy the same centrifugal force caused an increase in the minute volume from about 2.3 l/min in the pre-run period to an average of 12.0 l/min (Fig. 4).

Hence it seemed that vagotomy not only abolished some mechanism by which acceleration caused respiratory inhibition; it also "unmasked" some other mechanism by which acceleration greatly stimulated respiration. The magnitude of the hyperventilation seen in the majority of vagotomized animals as a result of even relatively mild gravitational stress in the head-to-tail direc-

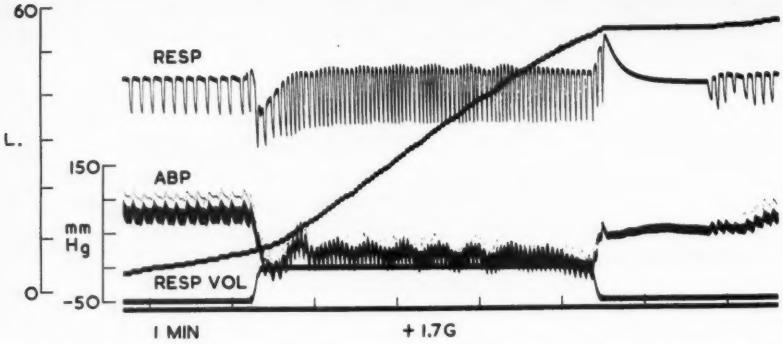

Fig. 4. Effects of prolonged positive acceleration. Same dog as in Fig. 2 and 3; breathing air, no external counterpressure to abdomen. Both vagus nerves previously cut; carotid sinus nerves intact. Recordings as in Fig. 2.

tion was impressive. The profound changes in the acid-base balance of the arterial blood which were associated with this hyperventilation are illustrated by the experiment depicted in Fig. 5, in which the respiratory minute volume increased from an average of about 4.0 l/min in the pre-run period to a maximum of about 19.0 l/min during exposure to 1.7 *g*. The arterial pH rapidly shifted to the alkaline side, showing a maximum change of about 0.35 of a pH unit at the end of the exposure.

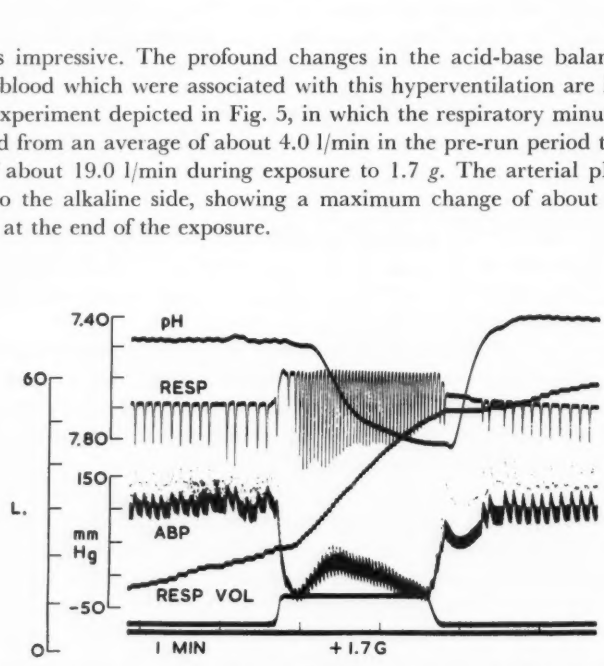

Fig. 5. Effects of prolonged positive acceleration. Dog 17.5 kg; morphine sulphate and chloralose; breathing room air, no external counterpressure to abdomen. Both vagus nerves previously cut, carotid sinus nerves intact. From above downwards: pH of arterial blood; otherwise as in Fig. 2. Note marked alkaline shift.

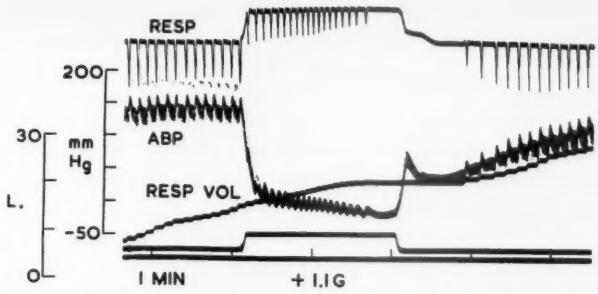

Fig. 6. Effects of prolonged positive acceleration. Same dog as in Fig. 5; breathing air, no external counterpressure applied to abdomen. Both vagus nerves cut, complete carotid denervation. Note development of respiratory failure towards end of run.

To investigate the mechanism of the hyperventilation observed in the vagotomized dogs and also in the post-apneic period in dogs with the vagi intact, the role of the carotid reflexogenic regions was now considered.

Effects of combined vagotomy and carotid denervation. As described above, arterial blood pressure at carotid sinus level always fell at the onset of a run, and although there was a secondary recovery when the vagi and the sinus nerves were intact, this recovery was never complete unless the run was discontinued. Since an increase in afferent impulse activity in the carotid chemoceptor fibres was demonstrated by LANDGREN and NEIL (1951) after the arterial blood pressure had been lowered by hemorrhage, and since positive acceleration also caused hypotension at the level of the carotid chemoceptors, the effects of carotid denervation were tested after previous vagotomy. This procedure invariably prevented the hyperventilation otherwise observed during centrifugation after previous vagotomy, and it sometimes resulted in the development of respiratory failure during a run (Fig. 5, 6). It can be concluded, therefore, that preservation of carotid chemoreflexes or baroreflexes, or both, were essential for the hyperventilation observed during positive radial acceleration after vagotomy.

It is essential that the respiratory failure seen in Fig. 6 towards the end of the run be distinguished from the initial period of apnea seen in the previous records. The initial apnea started immediately with the onset of a run; if the centrifuge were then stopped respiration was at once resumed. If, on the other hand, the run were continued, the apnea eventually gave way to respiratory movements. Finally, the initial apnea occurred only when the vagi were intact. By contrast, respiratory failure (as in Fig. 6) only developed some time after the onset of acceleration and was not terminated immediately by stopping the centrifuge. It was progressive and, if the run were continued, death followed

without resumption of respiration. Moreover, unlike the initial apnea, it could occur whether the vagi had previously been cut or not — indeed it was more likely to occur after vagotomy. Ischemic devitalization of the respiratory center was thought to be the most probable cause of this respiratory failure, for it was typically seen when the animals were exposed to higher *g* forces, or when vagotomy and section of the sinus nerves had seriously lowered the resistance of the cardiovascular system to gravitational stress.

Discussion

Moderate accelerative forces acting in the head-to-tail direction in the anesthetized dog produced a variety of changes in the pattern of respiration. To simplify analysis of what appeared to be a complex interplay of several mechanisms, attention was focussed on the two extremes of respiratory response: namely, the prolonged apnea observed in the initial phase of the runs, and the marked hyperventilation, seen particularly in vagotomized animals.

Mechanism of initial apnea. X-ray studies have shown that the abdominal and thoracic viscera are displaced caudally during positive acceleration (GAUER 1950). Hence it might be postulated that at the beginning of a run the usual efferent drive to the respiratory muscles was insufficient to produce respiratory movements against the increased load. Indeed, GAUER has shown in man that, as the centrifugal force is increased, ventilation is gradually shifted to the side of inspiration by the visceral displacement until finally respiration becomes impossible. But the initial apnea observed with the moderate *g* forces employed in the present experiments could not be explained in this way, because the apnea was shortened when the *g* force was increased. Furthermore, a long apnea was often followed by vigorous hyperventilation, again indicating little mechanical limitation of free respiratory movements. Nevertheless, the apnea was initiated by caudal displacement of the viscera since prevention of such displacement by the application of counterpressure to the abdomen shortened or abolished the apnea. Finally the effects of vagotomy (see below) seemed to establish a reflex basis for the apnea.

The effects of tilting on respiration are relevant in this connection. Thus REED and SCOTT (1949) observed in anesthetized dogs that respiration could be inhibited completely for several minutes by tilting to the head-up position during oxygen breathing. These authors also observed that vagotomy greatly reduced or abolished this response. Since in their experiments the tilting to a head-up position brought about a prolonged action of the normal force of gravity in the head-to-tail direction, and the running of the centrifuge in our experiments gave rise to a similar although greater force, also acting in the head-to-tail direction, the same mechanisms may be assumed to operate in the two cases. We agree with these authors that the apnea is probably due

mainly to activation of the Hering-Breuer reflex by caudal displacement of the diaphragm leading to expansion of the lungs and hence to stimulation of the pulmonary stretch receptors.

Mechanism of hyperventilation. Hyperventilation was observed during acceleration both before and after vagotomy; in the latter case it supervened without the occurrence of an initial period of apnea and was always more marked than with the vagi intact. With both vagi sectioned, hyperventilation during a run depended on the integrity of the carotid sinus nerves, indicating participation of chemoceptor and/or baroceptor mechanisms. Although decreasing the sinus pressure is known to give reflex stimulation of respiration (reviewed by HEYMANS and NEIL 1958) we think that increased chemoceptor drive is the most likely explanation for the hyperventilation observed in the present experiments.

In accepting chemoceptor excitation as responsible for the hyperventilation, the cause of such excitation that comes most immediately to mind is arterial hypoxemia. In fact hypoxemia (caused by venous admixture in the lungs) may develop during exposure to positive *g* loading despite concomitant hyperventilation and administration of 100 % O_2 (BARR, BJURSTEDT and COLERIDGE 1959). But low arterial O_2 cannot be the only stimulus to the chemoceptors, since reflex hyperventilation originating from the carotid bodies was more marked after vagotomy, when arterial desaturation did not develop.

However, it seems that chemoreflex hyperventilation in the absence of general arterial hypoxemia can occur also on the basis of localized hypoxia in the carotid bodies (ischemic excitation). Thus LANDGREN and NEIL (1951) attributed the intense chemoceptor discharge found in the carotid sinus nerve after hemorrhage to a reduction in blood flow through the carotid body; the activity of the chemoceptors became enormous when the mean blood pressure fell to about 50 mm Hg. Consequently, intense chemoceptor discharge would be expected in the present experiments when, as a result of positive acceleration even greater falls occurred in arterial pressure at the level of the carotid bodies. This is in harmony with earlier observations that respiratory changes, resulting from alterations in the intrasinusal pressure, may be of chemoreflex rather than baroreflex origin (EULER and LILJESTRAND 1937, BJURSTEDT and EULER 1942, BJURSTEDT and HESSER 1942). Moreover, LANDGREN and NEIL reported that the chemoceptor discharge, which appeared after hemorrhage during ventilation with room air, was greatly reduced by substitution of 100 % O_2. Analogous effects were observed in the present experiments; O_2 breathing (in animals with intact vagi) caused an impressive prolongation of the initial apnea, postponing the onset of hyperventilation, again suggesting chemoceptor rather than baroceptor control. Since the chemoceptors of the carotid sinus region were exhibited to a larger fall in arterial pressure than those of the aorta, the former would be expected to play the more important part in reflex control of respiration during positive acceleration.

Centrogenic factor. It has been claimed that the stimulating effect upon respiration of a fall in arterial blood pressure is not entirely caused by reflexes from the carotid and aortic regions, but is dependent also upon changes in blood flow which directly influence the activity of the respiratory center itself (reviewed by SCHMIDT 1941, p. 366). However, the results obtained after section of vagal and carotid sinus nerves suggested that such ischemic excitation of the respiratory center played no part in the hyperventilation observed in our experiments. Acceleration after chemoceptor denervation soon caused respiratory failure, indicating depression rather than excitation of the respiratory centre.

Interplay of factors. Thus during positive acceleration at least two antagonistic reflex mechanisms were seen to be operating, one tending to inhibit respiration and the other producing respiratory stimulation. The respiratory pattern in any particular run resulted from the interplay of these two mechanisms, one or other predominating with variations in the magnitude and the duration of the *g* load, the composition of the inspired gas etc. Hence progression from apnea to hyperventilation during a run depended on the inhibitory influence of pulmonary stretch reflexes being overcome in the course of time by an increasing chemoreflex drive; therefore breathing O_2 always prolonged the apnea. Again, the greater the *g* force, the greater the fall in arterial pressure, and the shorter was the period of apnea and the more likely was the apnea to be followed by hyperventilation.

Our results differed in several respects from the findings of previous workers. For various species (monkeys, dogs and cats — unanesthetized in most cases), BRITTON *et al.* (1947) reported that the respiratory rate was inhibited throughout "most" of the period (ranging from 10 sec to 6 min) of accelerative stress. But absence of details about effects produced by alterations in the duration and magnitude of the centrifugal force makes it difficult to comment on possible mechanisms responsible for the changes observed by these workers.

In the unanesthetized human subject the general experience is that exposure to moderate, positive *g* loadings causes increased ventilation (DIRINGSHOFEN 1934, GEMELLI 1936, GAUER 1938, LOMBARD, ROTH and DRURY 1948 and BROWNE 1958). This has been variously attributed to psychic factors and to arterial baroceptor influence; but in view of our present results we think that chemoreflex activity probably played a significant part in producing this increased ventilation.

The preponderance of inhibitory influences in the early stages of a run in anesthetized dogs is in striking contrast to these findings in conscious man. It is well recognized that anesthesia alters the balance between centrogenic and reflexogenic control of respiration in favour of the latter. For instance, in unanesthetized humans and dogs, continuous positive pressure respiration did not produce apnea (BOOTHBY and BERRY 1915); whereas CARR and ESSEX (1946) and BJURSTEDT (1953) found that the same procedure in anesthetized

dogs caused apnea which was abolished by vagotomy. Moreover CARR and ESSEX found that the apnea was considerably shortened if the anesthesia became light. Hence, if excitation of pulmonary stretch receptors is responsible for the respiratory inhibition, it must be assumed that this inhibitory effect is exaggerated by the general anesthesia (cf. WATROUS, DAVIES and ANDERSON 1950).

In conclusion, our experiments demonstrated a marked imbalance in the interplay of factors controlling pulmonary ventilation as the animals were exposed to moderately increased gravitational stress, acting in the head-to-tail direction for several minutes. This imbalance was characterized by abnormal predominance of reflex influence on the respiratory center, mainly from carotid chemoreflexes and pulmonary stretch reflexes. Such reflexogenic control often gained the upper hand to an extent which was apparently not compatible with the needs of the body for O_2 uptake and CO_2 removal.

We wish to express our appreciation to Mr. K. Berg, Mr. A. Lönn and other members of the Laboratory staff for their technical assistance.

Summary

1. Changes in the pulmonary ventilation of anesthetized dogs during positive radial acceleration (the force acting in the head-to-tail direction) were investigated using a large centrifuge. The accelerative force was kept sufficiently low (less than $+ 3$ *g*) to allow the animals to withstand exposures of several minutes duration without gross deterioration in their general condition.

2. With the vagi intact an initial apnea invariably occurred, followed by depressed, normal or increased ventilation as the run was continued at a constant *g* load.

3. The initial apnea was reduced or abolished by application of abdominal counterpressure and was always prevented by bilateral vagotomy. It is concluded that the apnea resulted from the inhibitory action of pulmonary stretch reflexes.

4. Evidence is presented that the hyperventilation observed during the runs was caused by an increased chemoreflex drive, resulting mainly from reduction in blood flow to carotid and aortic chemoceptors (ischemic excitation), and also in some cases from generalized hypoxemia secondary to venous admixture in the lungs.

5. The respiratory pattern in any particular run resulted from the interplay of these two mechanisms, one or other predominating with variations in the magnitude and the duration of the *g* load. Hence progression from apnea to hyperventilation during a run depended on the inhibitory influence of pulmonary stretch reflexes being overcome in the course of time by an increasing chemoreflex drive.

6. A striking feature of the respiratory response to positive radial acceleration was the prepotency of the reflexogenic control of respiration. This was exemplified by the fact that marked hyperventilation was regularly associated with gross respiratory alkalosis, indicating chemoreflex predominance.

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Acta physiol. scand. 1959. 47. 16—27

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Blood Gas Changes in the Anesthetized Dog during Prolonged Exposure to Positive Radial Acceleration

By

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Abstract

Barr, P.-O., H. Bjurstedt and J. C. G. Coleridge. Blood gas changes in the anesthetized dog during prolonged exposure to positive radial acceleration. *Acta physiol. scand.* 1959. *47.* 16—27. — Anesthetized dogs were exposed to increased gravitational stress in the head-to-tail direction and arterial O_2 saturation and acid-base balance changes studied. Simultaneous, direct and continuous recordings were made of arterial O_2 saturation and pH as well as respiratory minute volume in centrifuge runs. Application of moderate *g* forces over several minutes produced severe hypoxemia although 100 % O_2 was breathed and hyperventilation was present, indicating a great alveolar-arterial O_2 difference, and accordingly, a large intrapulmonary shunt.

Changes in the systemic circulation produced by the exaggerated gravitational stress often encountered in various flight maneuvers have been described (for reviews see Wood *et al.* 1946; Gauer 1950); but there is much less

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information about changes in the pulmonary circulation and gas exchange. That desaturation of arterial blood may develop during positive acceleration has been reported briefly (GAUER *et al.* 1949, HENRY 1950, HENRY *et al.* 1951).

Clearly, the last-mentioned observation is of great interest in that it draws attention to a hitherto unsuspected hazard which might be of importance in determining man's resistance to prolonged gravitational stress. Nevertheless, absence of information about concomitant changes in pulmonary ventilation makes it impossible to do more than speculate as to the cause of the fall in O_2 saturation.

The gross changes that may occur in pulmonary ventilation of anesthetized dogs during positive acceleration have been described in a previous paper (BARR, BJURSTEDT and COLERIDGE 1959). The experiments to be reported below were undertaken to study alterations in arterial O_2 saturation and acid-base balance under these conditions, and to determine to what extent such alterations may be explained by a) changes in total pulmonary ventilation, and b) local changes in the ventilation/perfusion ratios within the lungs, caused by the increased effective weight of the blood. A preliminary account of the findings has appeared (BARR, BJURSTEDT and COLERIDGE 1958).

Methods

Experiments were performed on 14 anesthetized dogs using a human centrifuge with the animal on a platform at one end of the double arm superstructure (for particulars, see BARR, BJURSTEDT and COLERIDGE 1959). The accelerative forces were kept sufficiently low (less than $+ 3\ g$) to allow exposures of several minutes duration without gross or irreversible deterioration in the general condition of the animals.

Recordings. Respiratory movements of the thorax, respiratory minute volume and arterial blood pressure at carotid sinus level were recorded.

The pH of the arterial blood was recorded continuously with a glass electrode assembly (BJURSTEDT, 1946) connected to the central end of the right femoral artery; the blood was returned to the femoral vein on the same side via a roller pump which gave a constant flow of 2 ml/sec. Oxygen saturation of the blood passing through the assembly was recorded continuously by means of a Millikan type double barrier cell oximeter coupled to a sensitive Multiflex galvanometer.

A ripple in the recording of arterial O_2 saturation occurred in some experiments (see *e. g.* Fig. 2); this was caused by the leads on the centrifuge arm passing through the magnetic fields around the driving motor for the centrifuge. This artefact was removed by balancing the currents from the "red" and "infra-red" cells against each other so as to cancel out the opposing ripples generated in the two circuits.

For further details of the methods employed in these experiments, see BARR, BJURSTEDT and COLERIDGE (1959).

Results and Discussion

The present investigation deals with certain relationships between 1) pulmonary ventilation, 2) arterial pH, 3) arterial O_2 saturation, and 4) local changes in the ventilation/perfusion ratios within the lungs.

In the pre-run periods arterial O_2 saturation was never complete, even when the animals were breathing 100 % O_2. This is in accordance with general experience for anesthetized dogs (*e. g.* WILLIAMS 1953) and is probably caused mainly by atelectasis which leads to the development of an intra-pulmonary right-to-left shunt. Arterial pH, which may be taken as reflecting the effective ventilation, ranged between 7.35 and 7.50 before any one run was started; in other words, anesthesia was never deep enough to cause a marked acidosis.

General hemo-respiratory reactions during positive radial acceleration. Positive radial acceleration markedly altered the pattern of respiration (BARR, BJURSTEDT and COLERIDGE 1959). Briefly, the changes were as follows: immediately after the onset of a run there was an initial apnea, followed by either depressed, normal or increased ventilation as the run was continued. Evidence has been presented that the initial apnea was caused by the inhibitory action of pulmonary stretch reflexes, whereas any tendency towards hyperventilation was caused mainly by predominance of chemoreflex drive secondary to stagnant anoxia in peripheral chemoceptors, or to general hypoxemia.

Changes in arterial O_2 and pH, and the relationship between these changes and concomitant alterations in pulmonary ventilation, presented a more complex picture. Thus, total ventilation during the runs was controlled mainly by reflex factors — blood gas changes appearing to exert only a minor influence. For example, hyperventilation persisted in spite of marked alkalosis in the absence of arterial hypoxemia, as after vagotomy (see below). Conversely, blood gas changes were not caused only by alterations in total ventilation and composition of the inspired gas. This was borne out by the fact that progressive arterial hypoxemia frequently developed during runs even when animals were hyperventilating on 100 % O_2. As will be deduced in more detail below, arterial blood gas changes were to a considerable extent produced by alterations in the total flow of blood through the lungs and by regional changes in ventilation/perfusion relationships within the lungs.

Arterial O_2 desaturation. The most pronounced fall in arterial O_2 saturation was observed when acceleration produced apnea or depressed respiration in animals breathing air. The maximum fall observed in these experiments, under air breathing and with a maximum duration of the applied *g*-force of 5 min, was one in which saturation fell from 95 to 60 %. Desaturation was usually progressive, sometimes with fluctuations associated with periodic respiration, as in Fig. 1. In this experiment there was an initial apnea starting immediately with the onset of the run, followed by depressed ventilation, the respiratory minute volume falling from about 3.0 l/min to 1.8 l/min. Arterial

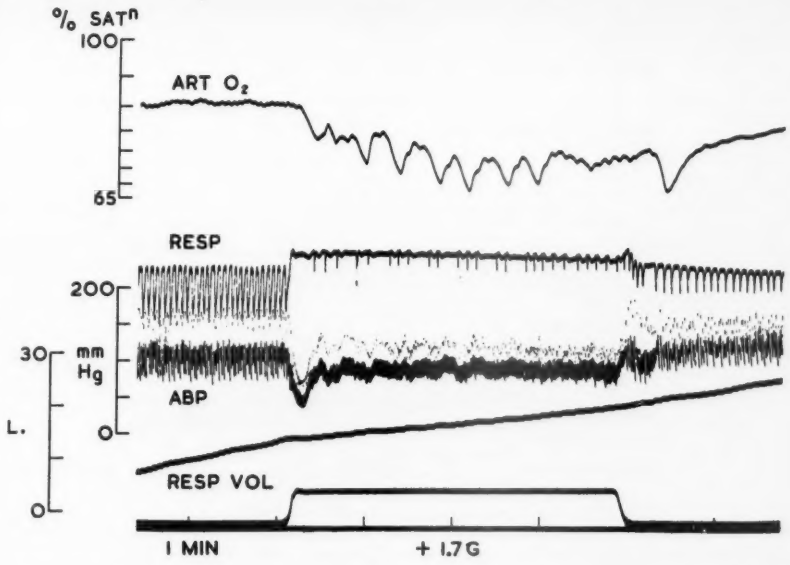

Fig. 1. Effects of prolonged positive acceleration (+ 1.7 *g*). Dog, 20 kg; morphine sulphate and chloralose; breathing air. Abdomen not supported by counterpressure; vagus and carotid sinus nerves intact. In this and the following figures from above downwards: O_2 saturation of the arterial blood (Art O_2); thoracic respiratory movements (Resp) inspiration downwards; arterial blood pressure (ABP) referred to the level of the carotid sinus; gas meter tracing (Resp vol) slope of which increases with the respiratory minute volume (calibration at the extreme left in liters, each step in the tracing represents an inspiration); accelerogram starting at and returning to zero *g* in the head-to-tail direction, plateau showing duration and magnitude of the positive acceleration as centrifuge is rotating. Time: 1 min between markings.

Note periodic respiration with reduction in rate and ventilation volume during run; also progressive desaturation with fluctuations corresponding to periodic respiration.

O_2 saturation fell from about 90 % to a minimum of about 67 %. The apparently close relationship between the periodic respiration and the fluctuations in arterial O_2 saturation might suggest that changes in oxygenation during the run were dependent upon concomitant changes in total pulmonary ventilation.

Nevertheless, that decreased respiratory activity was not the only factor responsible for the development of O_2 desaturation is seen in Fig. 2. In this experiment arterial O_2 saturation fell progressively from approximately 94 % to a minimum of about 83 %, despite the fact that respiratory minute volume had increased from about 4.0 l/min before the run to about 20.0 l/min by the end of the run — a fivefold increase; the respiratory rate had increased from about 12/min to 64/min. This development of desaturation while the animal

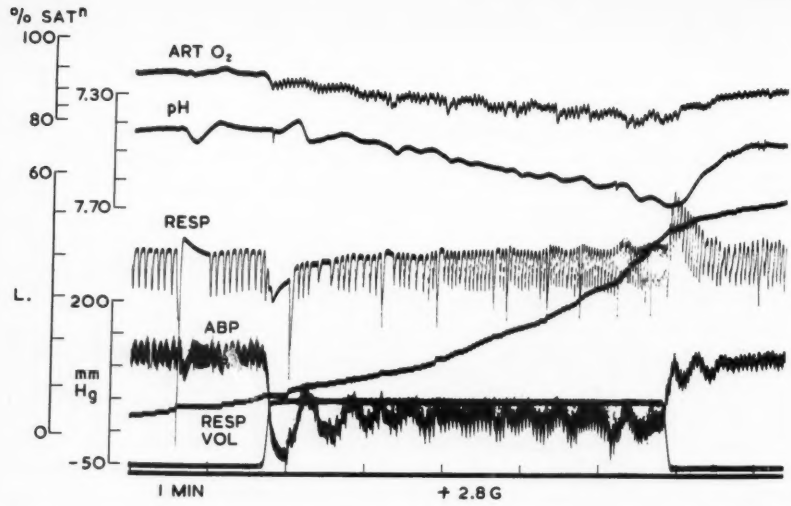

Fig. 2. Effects of prolonged positive acceleration (+ 2.8 *g*). Dog, 17.5 kg; chloralose; breathing 100 % O_2 before and during run, vagus and carotid sinus nerves intact. Recordings from above downwards: O_2 saturation of arterial blood; pH of arterial blood; otherwise as in Fig. 1.

Ripples in the recording of O_2 saturation during the run are artefacts — each ripple corresponds to one rotation of the centrifuge (see Methods). Descent of diaphragm reduced by external counterpressure to abdomen — hence there was only a brief apnea at the onset of acceleration. Note hyperventilation with development of progressive alkalosis and arterial O_2 desaturation, which demonstrates the development of a large pulmonary shunt. The dip in the arterial pH tracing one minute before the run was started corresponds to the spontaneous gasp shown by the stethograph tracing.

was hyperventilating on 100 % O_2 must point to the mixing of large amounts of venous with arterialized blood in the lungs.

Effects of vagotomy. A fall in arterial O_2 saturation was never observed in animals exposed to positive acceleration after bilateral cervical vagotomy (Fig. 3). This cannot be explained by the fact that vagotomy prevented the initial apnea; for desaturation during O_2 breathing occurred when the initial apnea was prevented by application of abdominal counterpressure, in animals with vagi intact. However, it was thought that the altered pattern of respiration after vagotomy might be of some importance in this connection. The marked hyperventilation seen during acceleration in vagotomized animals was due mainly to a large increase in the tidal volume and, although respiratory rate also increased, it never increased to the extent observed in dogs with intact vagi (Fig. 2 and 3). Consequently, for a given minute volume during acceleration, more effective alveolar ventilation would be obtained after vago-

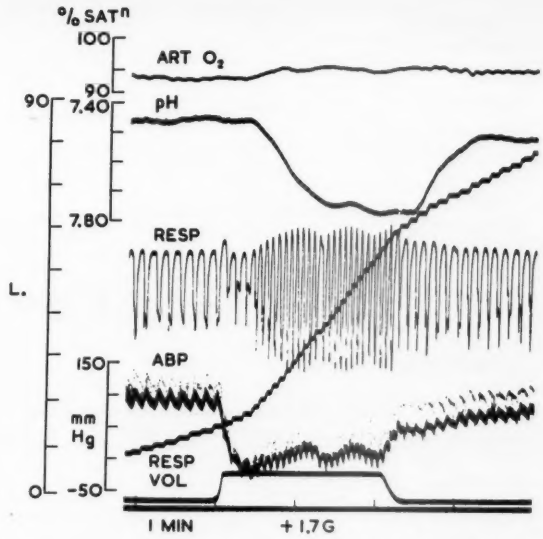

Fig. 3. Effects of prolonged positive acceleration (+ 1.7 *g*). Dog, 24 kg; morphine sulphate and chloralose, breathing air. Abdomen supported by counterpressure; both vagus nerves cut previously, carotid sinus nerves intact. Recordings as in Fig. 2.

Note development of marked alkalosis and hyperventilation during run. Vagotomy prevents desaturation.

tomy. Indeed, in the record shown in Fig. 3 (vagotomized dog breathing air) it can be seen that a slight increase in O_2 saturation occurred soon after the onset of the run.

However, further experiments proved this explanation to be inadequate; for, when the hyperventilation seen during runs after vagotomy was abolished by subsequent section of the carotid sinus nerves, acceleration still failed to elicit any fall in O_2 saturation (Fig. 5). Hence we are unable to offer an unequivocal explanation for this unexpected finding.

Nevertheless we wish to emphasize again that development of arterial desaturation during centrifugation cannot be attributed solely to alterations in total ventilation; for in animals breathing 100 % O_2 the mean alveolar pO_2 would still be in excess of that normally needed to maintain the original level of O_2 saturation — even with a complete cessation of respiratory movements for several minutes.

Persistence of desaturation in the post-run period. On stopping the centrifuge, a further fall in O_2 saturation sometimes occurred, lasting for some 10 to 15 sec. This additional fall was associated in some experiments with a brief respiratory pause (Fig. 1); in other experiments, however, it occurred in spite of

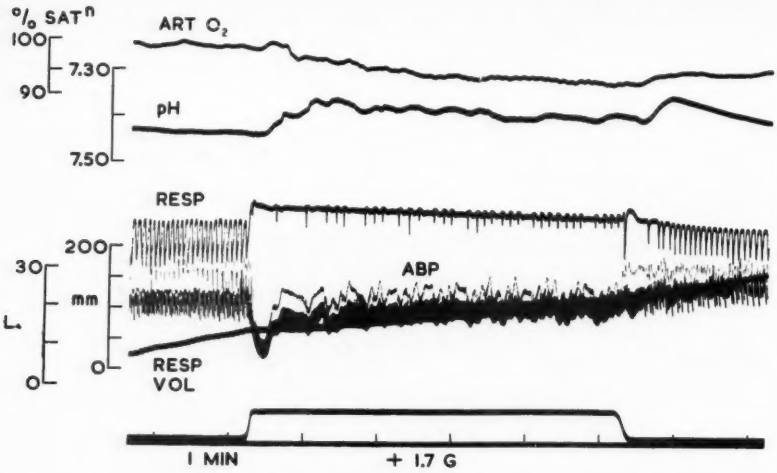

Fig. 4. Effects of prolonged positive acceleration (+ 1.7 *g*). Dog, 20 kg; chloralose, breathing air. Abdomen not supported by counterpressure; vagus and carotid sinus nerves intact. Recordings as in Fig. 2.

Note initial apnea followed by reduced ventilation; also fall in O_2 saturation and acid shift in pH of arterial blood.

continuing hyperventilation. The return of saturation after the runs towards the pre-run level was usually sluggish, even when the animal continued to hyperventilate on 100 % O_2. The time required for resaturation varied: in some cases the pre-run level was re-attained within the first minute; while in others resaturation occurred only after an interval of several minutes (Fig. 1 and 4) or was never complete. Hence a residual admixture of large amounts of venous to arterialized blood in the lungs was also present after the runs. The reason for this may be found in residual congestion and collapse of basal regions of the lungs observed post mortem by ourselves, and also by GAUER (1950).

Changes in arterial pH. As already stated, in the majority of experiments the onset of acceleration caused an immediate apnea which was shown to be dependent on pulmonary stretch reflexes. Therefore it was expected that, at the beginning of a run, the arterial pH would show a secondary change in the direction of a respiratory acidosis. In the event this was observed in some cases. For example in the experiment depicted in Fig. 2, the brief initial apnea was accompanied by a decrease in arterial pH; then, as respiration was resumed, the pH change was reversed and, with the onset of hyperventilation, there followed a marked shift to the alkaline side. In other experiments (*e. g.* Fig. 4), minute ventilation at the end of the run was still less than in the pre-run period;

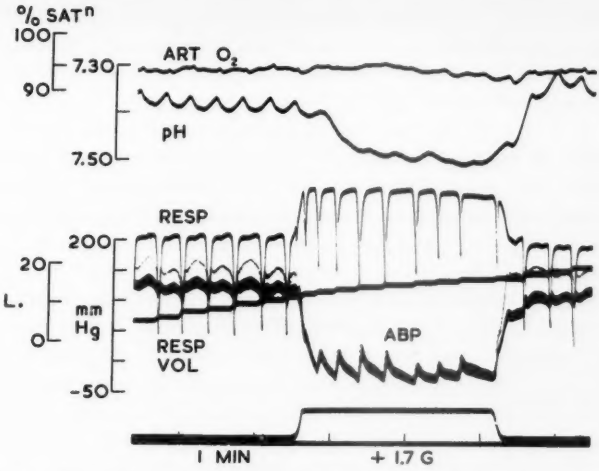

Fig. 5. Effects of prolonged positive acceleration (+ 1.7 *g*). Dog, 20 kg; chloralose, breathing O_2. Abdomen not supported by counterpressure; both vagus and carotid sinus nerves cut previously. Recordings as in Fig. 2.

Note little alteration in respiration during run; also marked shift in pH to alkaline side.

and, although the initial fall in pH was checked when respiration recommenced, the pH immediately before the end of the run was still to the acid side of the pre-run value.

In the majority of cases, however, alkalosis was the predominant feature, as in the experiments shown in Fig. 2 and 3. In general the most marked shifts in pH towards the alkaline side were observed in animals subjected to positive acceleration after bilateral vagotomy (Fig. 3). In this experiment respiratory minute volume increased from about 5.0 to 23.0 l/min during the run and arterial pH increased from 7.45 to 7.77.

From an examination of the results presented so far, exemplified by the records shown in Fig. 2, 3 and 4, it would seem reasonable to conclude that the changes observed in the pH of arterial blood during positive acceleration were secondary to alterations in total pulmonary ventilation. If so, in the experiment depicted in Fig. 4, the term "respiratory acidosis" might be used to describe the shift in pH towards the acid side found in combination with reduced ventilation. Conversely, "respiratory alkalosis" might well be used to describe the changes seen in Fig. 3 — the shift in pH being attributed to the observed increase in respiratory minute volume.

However, from an examination of further records, it became evident that concomitant variations in ventilation and pH during centrifugation were not invariably related as cause and effect. Although a shift in pH was often

associated with a marked degree of hyperventilation, the occurrence of hyperventilation was certainly not essential for development of alkalosis. For example, a slight shift towards the alkaline side was sometimes noted during an initial period of apnea. Again, in several experiments animals were exposed to positive acceleration after vagus and carotid sinus nerves had previously been sectioned to prevent ischemic excitation of the chemoceptive mechanisms (Fig. 5). Despite the fact that in these circumstances hyperventilation did not now occur during the run — indeed there was a small reduction in respiratory minute volume — arterial pH increased by approximately 0.1 pH unit.

Reduction of oxyhemoglobin may to a small extent have contributed to the alkalotic shifts through the HALDANE effect. However, the accelerative force must have acted through some additional mechanism to produce alkalosis, since it occurred also in the absence of both hyperventilation and hypoxemia, as shown in Fig. 5.

Conclusions

The simultaneous and continuous recordings of blood chemical changes and respiratory minute volume alterations allow certain unequivocal conclusions to be drawn from the results just described and discussed, at the same time demonstrating the rapid shifts in the interplay of various respiratory parameters that are caused by positive *g* loadings. The over-all picture was one in which reflexogenic control of respiration was predominant, as reported by BARR, BJURSTEDT and COLERIDGE (1959), and in which the observed changes in O_2 saturation and acid-base balance could not be explained solely as being secondary to alterations in the respiratory minute volume. In essence, the blood chemical changes indicate that profound disturbances in the pulmonary gas exchange may develop under prolonged *g* loadings. They will be further analyzed below for final conclusions, with reference to relevant observations that have been made by other investigators.

It seemed reasonable to expect that some disturbance of pulmonary function might result from changes in the pulmonary circulation during acceleration, since even normal gravity has been shown to change the ventilation/perfusion ratio within the subdivisions of the lung in the human (MARTIN, CLINE and MARSHALL 1955, INADA *et al.* 1954). It appears that the normal gravitational field produces an uneven distribution of blood flow favouring the dependent parts of the lung. The regional ventilation in the lobes of the dog lung is also affected by changes in posture and is reduced in the upper lobes when the animal is tilted from the supine to the upright position (RAHN *et al.* 1956). The net effect is, in brief, that the upper parts of the lung become relatively underperfused, while the dependent parts become relatively underventilated (reviewed by DRIPPS and SEVERINGHAUS 1955).

Furthermore, by means of X-ray studies (reviewed by GAUER 1950, p. 563) marked disturbances in the pulmonary circulation have been demonstrated to occur under positive *g* loadings. Thus, both in man and experimental animals, the lung apices appeared bloodless, while the basal parts of the lungs were congested.

It is apparent that the blood gas changes, which were observed in the present investigation, constitute a logical consequence of the above-mentioned effects of normal and increased gravitational forces. Our observation that hypoxemia occurred despite O_2 administration and a concomitant hyperventilation proves the development of a large shunt as a result of prolonged G loadings. This is in line with what might be expected from exposure of the lungs to strong gravitational fields: a manifold increase of the effective weight of the blood would cause a reduced participation of the apical alveoli in pulmonary gas exchange; conversely the hydrostatic pressure of the blood passing through the basal regions in the relatively unsupported and distensible pulmonary vascular bed might, if the *g* loading is of long duration, cause not only congestion but also the development of atelectasis.

In general, our results corroborate the concept that even normal gravity changes the ventilation/perfusion ratio within the subdivisions of the lung in dogs (RAHN *et al.* 1956) as well as in humans (MARTIN, CLINE and MARSHALL 1955, INADA *et al.* 1954), with the net effect that the upper parts of the lung become relatively underperfused, and the dependent parts relatively underventilated (reviewed by DRIPPS and SEVERINGHAUS 1955).

With respect to arterial pH, attention was paid to the fact that increased gravitational stress brought about arterial alkalosis (hypocapnia), even in the absence of hyperventilation. Since a sudden and gross reduction of tissue CO_2 production seems unlikely, evidence is in favour of a decreased transport of CO_2 to the lungs as the immediate cause of this type of arterial alkalosis. Some additional experiments were made under succinyl choline in which the respiratory minute volume was kept constant by means of a STARLING pump. It was observed that positive *g* loadings as well as hemorrhage alone, both causing a decrease of the return of venous blood to the heart and consequently a diminished total perfusion of the lungs, caused a shift of the arterial pH in the alkaline direction. The observations under hemorrhage, which were made on dogs and will be treated in a subsequent paper, support the concept that prolonged *g* loadings may bring about arterial alkalosis through peripheral retention of CO_2, secondary to diminished cardiac output.

We wish to express our appreciation to Mr. K. BERG, Mr. A. LÖNN and other members of the Laboratory staff for their technical assistance.

Summary

1. Experiments were performed on anesthetized dogs to determine the nature of such alterations in arterial O_2 saturation and acid-base balance that may occur during prolonged exposures to increased gravitational stress (force acting in the head-to-tail direction). For this purpose the animals were spun in a large centrifuge, arterial O_2 saturation and pH, as well as respiratory minute volume and arterial blood pressure being recorded.

2. The arterial O_2 saturation fell more during the runs than could be explained by changes in ventilation and pO_2 of the inhaled gas mixtures. Likewise, there was a greater tendency for arterial pH to shift towards the alkaline side than could be explained on the basis of ventilatory changes.

3. The progressive arterial O_2 desaturation observed during the centrifuge runs must be conceived as resulting in part from the mixing of large amounts of venous to arterialized blood in the lungs (intrapulmonary shunting effect), since it occurred in spite of inhalation of 100 % O_2 and concomitant hyperventilation. Such desaturation did not occur after previous bilateral vagotomy.

4. Evidence is presented that the arterial alkalosis (hypocapnia), which may develop during prolonged *g* loadings and become very marked when hyperventilation is present, may be caused in part by peripheral retention of CO_2, secondary to a diminished cardiac output.

5. The observed progressive blood chemical changes are interpreted in terms of changes in regional and over-all ventilation/perfusion ratios in the lungs.

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Acta physiol. scand. 1959. 47. 28—43

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Effects of Raised Barometric Pressures on Respiration in Man[1]

By

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Abstract

Hesser, C. M. and B. Holmgren. Effects of raised barometric pressures on respiration in man. *Acta physiol. scand.* 1959. *47.* 28—43. — The effects of raised barometric pressures (up to 4.0 atm) on various respiratory functions, hitherto largely neglected, were studied on 8 healthy subjects at rest in a recompression chamber. The independent effects of changes in inspired oxygen and nitrogen pressures were studied by comparing data obtained on air, 100 % oxygen, and 5 % oxygen in nitrogen at various ambient pressures. Breathing air with increasing ambient pressure, respiration became progressively slower and deeper, whereas at 4.0 atm the effective alveolar ventilation was slightly increased. Other observations at 4.0 atm were: Average increase of 33 % in tidal volume; average decreases in respiratory rate of 27 %, respiratory minute volume of 10 %, and functional dead space/tidal volume ratio of 9 %; no demonstrable changes in expiratory reserve volume, functional dead space and respiratory exchange ratio. Evidence is presented that the respiratory changes were caused by the combined effects of increased oxygen tension and of increased breathing resistance due to increased gas density. Thus, nitrogen at high pressures (up to 3.8 atm) exerted little, if any, depressant action on respiration.

[1] The symbols used in this paper conform to the standards published in *Fed. Proc.* 1950. *9.* 602—605.

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Apart from the effects on the alveolar gas composition (see HALDANE and PRIESTLEY 1935, p. 32) no systematic investigations seem to have been published as to the effects of raised barometric pressures on respiration in man during air breathing at rest.

At elevated pressures various respiratory functions may, however, be expected to become modified as a result of changes in at least three physical properties of the inhaled air, *viz.* 1) increased oxygen pressure, 2) increased nitrogen pressure, and 3) increased gas density. The present observations refer to respiratory rate and minute volume as well as calculated data of alveolar ventilation, respiratory exchange ratio, and dead space components in 8 healthy subjects breathing O_2—N_2 gas mixtures at various ambient pressures up to 4.0 atmospheres absolute (atm). The high N_2 pressure effects were studied by supplying a low O_2 per cent (to maintain a normal O_2 tension) at increased ambient pressures. The significance of the changes observed has been evaluated by statistical analysis.

Subjects, Methods and Procedure

Subjects. The ages, body measurements, resting metabolic rates and vital capacities of the 8 healthy male subjects in this study are given in Table I. All were well acquainted with the sensations of compression and decompression, seven were professional or amateur divers.

Methods. The subject and one of the investigators were exposed to the same ambient pressure in a large recompression chamber. Visual and auditory communications were established between the observers outside and inside the steel chamber. The chamber temperature ranged from 21° to 27° C during the test periods of all experiments, the maximum variation over a single day being $\pm$ 2° C. Pressure variations within the chamber during the test periods did not exceed $\pm$ 3 mm Hg, the chamber pressure (in excess of barometric pressure) being read on a differential Hg-manometer. Barometric pressure averaged 760.6 mm Hg (range 744.0—771.0). The duration of each experiment ranged from 5 to 6 hours.

Table I. Vital Statistics of Eight Subjects Studied

Subject	Age years	Weight kg	Height cm	BMR ml O_2 per min, STPD	Vital Capacity lit. BTPS
DB	25	75	188	264	5.46
IS	24	60	181	200	4.04
BL	22	58	173	200	4.65
BC	38	74	179	240	4.69
GL	30	66	172	231	4.52
SS	36	71	164	230	4.10
GF	35	89	187	273	5.91
BH	35	65	172	202	4.60

Commercial compressed air, 100 per cent oxygen, or 5.06—5.47 per cent oxygen in nitrogen (hereafter called 5 % O_2) were administered at normal and/or at raised barometric pressures by means of a respiratory system comprising a mouthpiece, nose clip, a special low resistance and low dead space (about 10 ml) breathing valve assembly (v. DÖBELN 1949), and three 100-liter vinyl plastic Douglas bags (PERKINS 1954) inside the chamber, each bag connected to a high pressure cylinder placed outside. The expired air was delivered to a calibrated gas meter located inside the chamber, via a 3-way stopcock and a smooth bore rubber hose of 1.7 meter length and 2.3 cm inside diameter. The total resistance (cm of water) of this system at various flows and at various ambient pressures was as follows:

Flow l/sec	1 atm		2 atm		4 atm	
	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.
0.3	0.8	1.2	1.0	1.4	1.4	1.8
0.5	1.1	1.7	1.5	2.1	2.4	3.2
1.0	2.2	3.2	3.2	5.1	6.2	9.9

Because of the possibility that the respiratory functions at a given pressure may be influenced by the preceding pressure, two series of experiments were performed on each subject, "Series I" with successive, stepwise increments in pressure from 1.0 atm, "Series II" with successive, stepwise decrements in pressure from 4.0 atm. The ambient pressures and gas mixtures used were:

Series I. 1.0 atm: Air and 100 % O_2; 2.0 atm: Air; 3.0 atm: Air and 5 % O_2; 4.0 atm: Air and 5 % O_2; (1.9 atm: Air); 1.6 atm: 100 % O_2; 1.3 atm: Air; 1.0 atm: Air.

Series II. 1.0 atm: Air and 100 % O_2; 4.0 atm: Air and 5 % O_2; 3.0 atm: Air and 5 % O_2; 2 atm: Air; 1.6 atm: 100 % O_2; 1.3 atm: Air; 1.0 atm: Air.

The two series were performed with an interval of 3 to 12 days (in one case 70 days). On the first experimental day 5 subjects performed Series I and the remainder Series II, control data obtained by beginning and ending with air breathing at 1.0 atm (sea level pressure). The results obtained in the control experiments showed no statistically significant differences and were therefore averaged for each subject. Nor was there any significant difference between results obtained in the two series under the same ambient conditions. Accordingly, these data were also averaged for each subject before comparison with control data.

Before either series was started on any one subject, his resting metabolic rate and vital capacity were measured by means of a Krogh spirometer filled with oxygen. The rate of oxygen consumption was measured over a 10-min period after the subject had rested for at least 30 min.

Experimental Procedure. Since observations at higher pressures may be influenced by conditions which are not significant at 1.0 atm, the procedure is described in detail.

After a light breakfast the subjects were indoctrinated during the first 15 min of a 45 min rest period, which was spent in a semi-recumbent position. At least 8 min were allowed to pass after each change in ambient pressure or of inhaled gas mixture before the respiratory rate and minute volume were determined over a 5 to 6 min test period. From these measurements the individual average tidal volumes were calculated. The tests began and were timed from the end of a normal expiration, simultaneously the subject turned the 3-way stopcock to deliver the expired air to the gas meter. After a timed 5 to 6 min, the subject turned back the stopcock, again at the

end of a normal expiration. The chamber temperature and pressure, immediately before and after each test, were recorded.

In the experiments at 1.0 and 4.0 atm samples of alveolar gas were collected about two minutes later by means of the Haldane-Priestley technique, *i. e.* after a normal expiration the subject made a forced expiration into the gas meter, trapping the expelled expiratory reserve volume by turning the stopcock. Samples of this gas were drawn from a tap near the stopcock into Hg tonometers and stored under positive pressure until analyzed in duplicate at sea level pressure for oxygen and carbon dioxide with a modified Haldane apparatus.

Procedure Used for Interpretation of Data. The data obtained have been compared mainly in the following ways.

Comparison I. By comparing data obtained during *air* breathing at various raised barometric pressures the effects of simultaneous changes in inspired P_{O_2}, inspired P_{N_2}, and in gas density were evaluated.

Comparison II. The effects of an increase in inspired P_{O_2} (from 149 to 714 mm Hg) at a *low* P_{N_2} level were estimated by comparing data obtained on oxygen and on air at 1.0 atm.

Comparison III. By comparing data on air and on 5 % O_2 in N_2 at 4.0 atm the effects of an increase in inspired P_{O_2} (from 153 to 610 mm Hg) at a *high* P_{N_2} level were determined.

Comparison IV. The isolated effects of an increase in inspired P_{N_2} of about 3.0 atm (and/or in gas density) at a *normal* inspired P_{O_2} were evaluated by comparing data from experiments on 5 % O_2 in N_2 at 4.0 atm with data on air at 1.0 atm.

Comparison V. By comparing data on air at 4.0 atm with data on oxygen at 1.0 atm the effects of increasing the inspired P_{N_2} by about 3.0 atm at a *high* inspired P_{O_2} level were determined.

The statistical significance of the observed differences in these five comparisons was evaluated by analyzing the differences in the single individuals using the t-test (see *e. g.* FISHER 1948). Thus every subject served as his own control.

Results

The means and standard deviations of measured and calculated values of various respiratory functions in eight normal, resting subjects while breathing air, 100 per cent oxygen, or 5 per cent oxygen in nitrogen at normal and/or at various raised barometric pressures (up to 4.0 atm) are shown in Tables II and III. A statistical evaluation of the differences observed is provided in Table IV. Individual and mean values of respiratory minute volume, respiratory rate, and tidal volume during air breathing at 1.0, 1.3, 2.0, 3.0 and 4.0 atm are plotted in Fig. 1, *A*, *B* and *C*. The same respiratory functions, expressed in per cent of the respective control values (air breathing at sea level pressure) are shown in Fig. 1 *D*.

Respiratory Minute Volume. With increasing ambient pressure the average value of respiratory minute volume ($\dot{V}_E$ l/min BTPS) showed a continuous decrease from 6.46 l (1.0 atm) to 5.73 l (3.0 atm), and thereafter a slight increase to 5.82 l (4.0 atm) (Table II and Fig. 1, *A* and *D*). The 11 per cent decrease at 3.0 atm is highly significant ($P < 0.001$). A significant reduction in $\dot{V}_E$ of approximately the same magnitude was also induced by an isolated

Table II. Respiratory Effects of Air, 100 % O_2 and 5 % O_2 in N_2 at Various Ambient Pressures

(Mean Values. Standard Deviations in brackets.)

Inspired Gas		Air					Oxygen		5 % O_2 in N_2	
Ambient Pressure, atm. absolute		1.0	1.3	2.0	3.0	4.0	1.0	1.6	3.0	4.0
P_B—47, mm Hg		714	924	1,437	2,177	2,914	714	1,146	2,176	2,914
Inspired P_{O_2}, mm Hg		149	193	301	456	610	714	1,146	114	153
Inspired P_{N_2}, mm Hg		565	731	1,136	1,721	2,304	—	—	2,062	2,761
Number of Subjects		8	8	8	8	8	8	8	6	8
Resp. Minute Volume	l/min at BTPS	6.46 (0.76)	6.14 (0.69)	6.05 (1.10)	5.73 (0.91)	5.82 (0.77)	6.42 (1.35)	6.49 (1.16)	5.98 (0.74)	5.77 (0.85)
	Per cent of control[1]	100	95.4 (6.3)	93.1 (7.8)	88.5 (6.0)	90.3 (7.6)	98.7 (10.4)	100.2 (10.3)	91.6 (7.8)	89.3 (6.4)
Resp. Rate	Breaths per min	9.60 (3.71)	9.21 (3.73)	8.65 (3.93)	7.73 (3.43)	6.89 (3.48)	8.65 (3.71)	8.81 (3.75)	8.47 (3.71)	7.73 (3.97)
	Per cent of control[1]	100	95.7 (11.6)	88.4 (13.9)	80.5 (16.4)	72.8 (22.9)	90.0 (13.1)	91.1 (12.6)	85.9 (21.9)	79.9 (23.4)
Tidal Volume	ml at BTPS	808 (411)	801 (384)	868 (486)	895 (396)	1,019 (417)	893 (419)	885 (439)	869 (533)	964 (505)
	Per cent of control[1]	100	99.9 (9.6)	105.4 (10.3)	113.2 (19.9)	133.1 (44.7)	111.7 (17.7)	110.0 (15.1)	109.9 (21.6)	121.1 (44.5)

[1] Control = Air breathing at sea level pressure.

increase in P_{N_2} (*Comparison IV*), whereas there were no significant changes (Table IV) by an isolated increase in P_{O_2} (*Comparison II and III*).

Respiratory Rate. With one exception (subject I. S.) the respiratory rate (f) decreased with increasing ambient pressure, the average decrease bearing an approximately linear relationship to the ambient pressure and amounting to 27 per cent at 4.0 atm (Table II and Fig. 1, *B* and *D*). The reductions of respiratory rate at 3.0 and 4.0 atm represent significant changes when compared with data obtained at 1.0 and at 1.3 atm. An increase of either the inspired P_{O_2} (*Comparison II and III*) or the inspired P_{N_2} (*Comparison IV and V*) also caused a reduction of the respiratory rate (Table IV).

Tidal Volume. The continuous reductions in f and $\dot{V}_E$ with increasing ambient pressure were accompanied by a continuous increase in tidal volume (V_T ml BTPS), the average increase amounting to 33 per cent at 4.0 atm (Table II and Fig. 1, *C* and *D*). At 3.0 and 4.0 atm V_T was significantly higher than at 1.3 atm ($P < 0.01$). V_T showed a tendency to increase also when either the inspired P_{O_2} (*Comparison II and III*) or the inspired P_{N_2} (*Comparison IV and V*) was increased (Table IV).

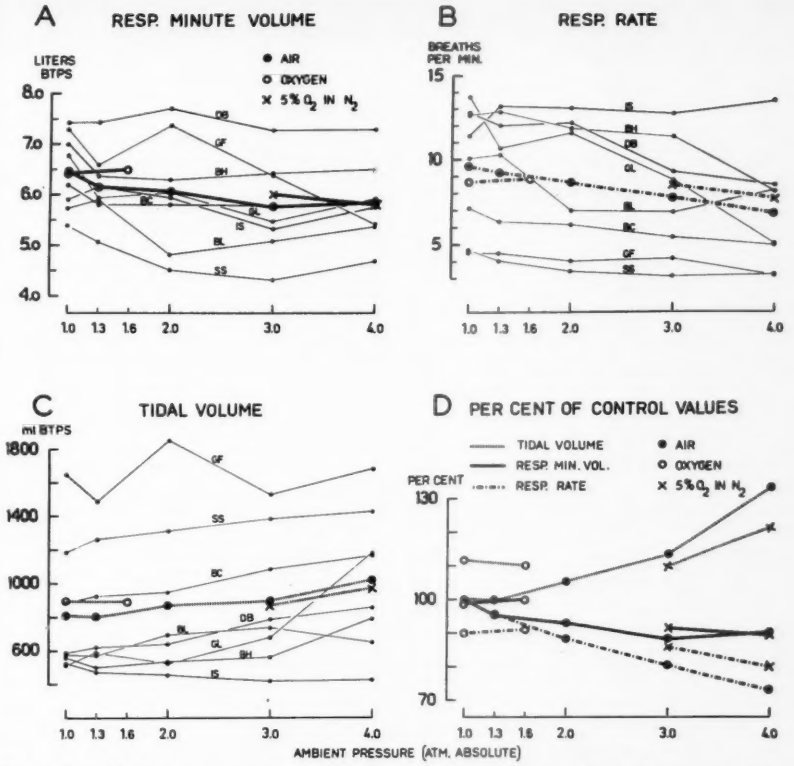

Fig. 1. Individual and mean values of respiratory minute volume (*A*), respiratory rate (*B*), and tidal volume (*C*) during inhalation of air (●), 100 % O_2 (○), and 5 % O_2 in N_2 (×) at various ambient pressures. Each point in the graphs connected by thin solid lines represents the average of two determinations (Series I and Series II) on individual subjects breathing air. Mean values are connected by heavy lines. The same respiratory functions in per cent of the respective control values (air breathing at sea level) are shown in *D* (mean values).

Expiratory Reserve Volume. The expiratory reserve volume showed no significant change when the ambient pressure was raised to 4.0 atm (Table III). This observation, together with the fact that the tidal volume increased as the ambient pressure was raised, suggests that the lung volume at the mid-respiratory level increased with increasing ambient pressure.

Respiratory Exchange Ratio. The respiratory exchange ratio (R) during air breathing at 1.0 and 4.0 atm was calculated for each subject by the following expression of the alveolar gas equation (RAHN and FENN 1955, p. 37):

$$R = \frac{P_{A_{CO_2}} (1 - F_{I_{O_2}})}{P_{I_{O_2}} - P_{A_{O_2}} - F_{I_{O_2}} \times P_{A_{CO_2}}} \qquad (1)$$

Table III. Alveolar P_{CO_2} and P_{O_2}, Expiratory Reserve Volume, and Calculated Values of Respiratory Exchange Ratio, Alveolar Ventilation and Functional Dead Space at Various Inspired P_{O_2} and P_{N_2}

(Mean Values. Standard Deviations in brackets.)

Inspired Gas		Air		Oxygen	5 % O_2 in N_2
Ambient Pressure, atm absolute		1.0	4.0	1.0	4.0
Inspired P_{O_2}, mm Hg		149.4 (1.0)	609.8 (1.0)	714 (4.8)	153.1 (5.7)
Inspired P_{N_2}, mm Hg		565	2,304	—	2,761
Number of Subjects		8	8	8	8
Expir. Reserve Volume, litres at BTPS		1.43 (0.15)	1.40 (0.17)	1.38 (0.16)	1.43 (0.30)
Alveolar P_{CO_2}, mm Hg		40.5 (2.8)	39.7 (2.6)	38.3 (2.5)	41.1 (2.8)
Alveolar P_{O_2}, mm Hg		102.1 (5.3)	563.0 (6.2)	[1]676	108.0 (5.5)
Respir. Exchange Ratio[2]		0.830 (0.064)	0.829 (0.088)	—	—
Alveolar Ventilation	l/min at BTPS[3]	4.09 (0.69)	4.16 (0.59)	4.33 (0.73)	4.03 (0.60)
	Per cent of control[4]	100	102.0 (4.3)	105.9 (3.2)	98.6 (3.4)
Functional Dead Space	ml at BTBS[5]	260 (85)	230 (159)	248 (123)	231 (109)
	Per cent of Tidal Volume	36.1 (11.6)	27.2 (16.6)	31.0 (13.7)	29.1 (14.1)

[1] Assumed 38 mm Hg less than inspired P_{O_2}.
[2] Calculated by Equation 1 (see text).
[3] Calculated by Equation 2 (see text).
[4] Control = Air breathing at sea level pressure.
[5] Calculated as (Resp. Min. Vol. — Alv. Ventil.)/Resp. Rate.

Results of these calculations show that R was not measurably altered when the ambient pressure was raised to 4.0 atm, the average values of R amounting to 0.830 and 0.829 at 1.0 and 4.0 atm, respectively (Table III).

The alveolar gas, obtained by the Haldane-Priestley end-expiratory technique, was probably not quite representative for mean alveolar air, but yielded values that were too high in CO_2, and too low in O_2 and in R (RAHN 1954). At raised barometric pressures the discrepancy should become greater as a result of the observed reduction in respiratory rate. Since equation 1 is valid only for conditions in which the inspired N_2 volume is equal to the expired N_2 volume, another error is introduced when using equation 1 for calculation of R in our experiments at 4.0 atm. From published data on the N_2 exchange rate of the human body (JONES 1950, BEHNKE 1951, LUNDIN 1953) we have

Table IV. Comparisons of Respiratory Parameters under Various Ambient Conditions (8 Subjects)

Parameter	Differences in Comparison No.				
	I	II	III	IV	V
	Air, 4.0 atm to Air, 1.0 atm	O_2, 1.0 atm to Air, 1.0 atm	Air, 4.0 atm to 5 % O_2, 4.0 atm	5 % O_2, 4.0 atm to Air, 1.0 atm	Air, 4.0 atm to O_2, 1.0 atm
Inspired P_{O_2}, mm Hg....	461	565	457	4	— 104
Inspired P_{N_2}, mm Hg....	1,739	— 565	— 457	2,196	2,304
Resp. Rate, Breaths per min.....	— 2.71*	—0.95	— 0.84	— 1.87	— 1.76
Tidal Volume, ml at BTPS	211*	85	55	156	126
Resp. Min. Volume, l/min at BTPS.....	— 0.64*	—0.04	0.05	— 0.69**	— 0.60
Alv. Ventil., l/min at BTPS	0.07	0.24**	0.13*	— 0.06	— 0.17*
Functional Dead Space, ml at BTPS.....	— 30	— 12	— 1	— 29	— 18
Functional Dead Space in per cent of Tidal Vol..	— 8.9**	— 5.1*	— 1.9	— 7.0**	— 3.8

* Probably significant difference ($0.05>P>0.01$)

** Statistically significant difference ($P<0.01$)

Significance of differences based on analysis by the t-test of differences in the single individuals (df=7) (see FISHER 1948).

calculated that in Series II at 4.0 atm, which involved the greatest change in P_{N_2}, the rate of N_2 uptake from the lungs at the time of collecting alveolar gas would be about 30 ml STPD per min. It can be estimated then, that, due to the N_2 uptake, the calculated R values of the experiments on air at 4.0 atm were about 0.03 too high. Thus the two errors introduced when applying equation 1 for calculation of R in the experiments at 4.0 atm tend to nullify each other. We therefore feel justified in using the calculated R values for comparative studies.

Alveolar Ventilation. The observation that $\dot{V}_E$ decreased with increasing ambient pressure does not necessarily indicate that the effective alveolar ventilation ($\dot{V}_A$ l/min BTPS) also diminished, since the simultaneous increase in tidal volume and changes in functional dead space could have compensated for the reduction in $\dot{V}_E$.

To calculate $\dot{V}_A$ in the experiments at 1.0 and 4.0 atm we have used the following expression of the alveolar ventilation equation (RAHN and FENN 1955, p. 37):

$$\dot{V}_A = \frac{0.863 \times \dot{V}_{CO_2}}{P_{A_{CO_2}}} \quad (2)$$

The rate of CO_2 output ($\dot{V}_{CO_2}$ ml/min STPD) was assumed to be the same in the various experimental conditions and equal to the product $\dot{V}_{O_2} \times R$ obtained at 1.0 atm. This assumption was based on the observations of LAMBERTSEN *et al.* (1953 c), who found no significant change in $\dot{V}_{CO_2}$ when the ambient pressure was increased to 3.5 atm. Furthermore, our findings that R was not measurably altered as the ambient pressure was raised to 4.0 atm suggest that no important interference with the CO_2 output occurred at this pressure.

$\dot{V}_A$ was calculated for each subject and the means are presented in Table III. These values are probably somewhat smaller than the true values since, as mentioned before, the alveolar samples obtained yielded values that were too high in CO_2, and too low in R. In the experiments at 4.0 atm the true values of $\dot{V}_A$ should be somewhat larger also as consequence of the increased air density causing increased work of breathing and, therefore, an increase of resting $\dot{V}_{O_2}$. However, the errors in the calculated $\dot{V}_A$ values should be of no significance when comparing data obtained at the same ambient pressure.

Although increase of the inspired P_{O_2} at a low or high P_{N_2} level (*Comparison II and III*) caused no consistent change in $\dot{V}_E$, there was a slight but significant increase of $\dot{V}_A$ (5.9 % and 3.4 %, respectively). Increasing the inspired P_{N_2} by about 3.0 atm caused, on the other hand, a reduction in both $\dot{V}_E$ and $\dot{V}_A$ (*Comparison IV and V*).

When the inspired oxygen and nitrogen presures were increased simultaneously by raising the ambient pressure during air breathing from 1.0 to 4.0 atm, $\dot{V}_A$ increased somewhat (*Comparison I*). The significant reduction in $\dot{V}_E$ at 4.0 atm was thus accompanied by an increase rather than by a decrease in $\dot{V}_A$. The true increase in $\dot{V}_A$ should be larger than the calculated average increase of only 2.0 per cent since the calculated values of $\dot{V}_A$ at 4.0 atm probably were too small in relation to those at 1.0 atm.

Functional Dead Space. Functional dead space (V_D) was calculated as $(\dot{V}_E - \dot{V}_A)/f$ for each subject. The means of these calculations are shown in Table III, and expressed in ml BTPS as well as in per cent of corresponding tidal volumes. The values recorded are probably too high since, as mentioned above, the calculated values of $\dot{V}_A$ most likely are somewhat too small. The V_D errors should be rather small, however, and be of no significance for the comparative studies.

As shown by Table IV there were no significant differences between the absolute values of V_D in any of the experimental conditions studied. On the other hand V_D/V_T showed a significant decrease from 0.36 to 0.27 when,

during air breathing, the ambient pressure was raised from 1.0 to 4.0 atm. An increase of either the inspired P_{O_2} (*Comparison II and III*) or the inspired P_{N_2} (*Comparison IV and V*) was also followed by a decrease in this fraction. Thus the fraction amounted to 0.31 during oxygen breathing at 1.0 atm, and to 0.29 during inhalation of 5 % O_2 at 4.0 atm. Both values are significantly less than the control value (air breathing at 1.0 atm).

Discussion

The present investigation shows that, during air breathing at rest, respiration in man is affected in several ways by raised barometric pressures. Since an increase of the ambient pressure involves simultaneous increases of the inspired P_{O_2}, inspired P_{N_2}, and the gas density, the respiratory changes observed may have been caused by either of these factors or by a combination of them. The effects of one factor may also have masked the effects of any of the two others. *Comparison I*, therefore, cannot be used to differentiate or evaluate the various factors involved. However, by comparing data from experiments in which only the inspired P_{O_2} (*Comparison II and III*), or the inspired P_{N_2} and gas density (*Comparison IV and V*) were increased, the isolated effects of these factors could be estimated. The results of these comparisons will first be discussed.

Effects of Changes in Inspired P_{O_2}. An increase of the inspired P_{O_2} caused a significant increase in $\dot{V}_A$, a slight increase in V_T, no demonstrable changes in $\dot{V}_E$ and V_D, and a decrease in f and V_D/V_T. This was the case when P_{O_2} was increased by 565 mm Hg at a *low* P_{N_2} level (*Comparison II*), and when increased by 457 mm Hg at a *high* P_{N_2} level (*Comparison III*).

The increase of $\dot{V}_A$ as demonstrated in *Comparison II* confirms earlier observations that inhalation of oxygen by normal men at sea level for periods exceeding 5—10 minutes produces a slight increase of the respiratory activity (Dautrebande and Haldane 1921, Lambertsen *et al.* 1953 a, Shephard 1955). Our results indicate that this increase may not be revealed if only $\dot{V}_E$ is measured, and, furthermore, as shown by *Comparison III*, that the respiratory stimulation induced by increasing the inspired P_{O_2} to 600 mm Hg or more is not appreciably affected by high nitrogen pressures or by raised barometric pressures up to at least 4.0 atm.

Concerning the effects of oxygen inhalation on the pattern of respiration there is a lack of unanimity among earlier reports, which may be attributable to various influences such as differences in the duration of and the techniques used in the O_2 administration (Bean 1945). Although according to our results (*Comparison II*) respiration tends to become slower and deeper during oxygen breathing, the number of observations is not sufficient to establish the significance of these changes. As shown by *Comparison III* similar changes occurred

when the O_2 tension was increased at a N_2 pressure of about 3.5 atm, which suggests that high N_2 pressures do not to any appreciable degree modify the effects of high O_2 tensions on the respiratory pattern.

Our findings that an increase of the inhaled O_2 tension did not alter the absolute volume of the functional dead space are supported by the observations of PAPPENHEIMER, FISHMAN and BORRERO (1952), who found the numerical value of the effective dead space to be independent of the inspired O_2 tension. According to *Comparison III* this is true also at high N_2 pressures.

As a consequence of no change in V_D and an increase in V_T, the fraction V_D/V_T decreased as the inhaled O_2 tension was raised by about 500 mm Hg. This was the case at a low as well as at a high P_{N_2} level.

Effects of Changes in Inspired P_{N_2} and Gas Density. Increasing the inspired P_{N_2} by about 3.0 atm, at normal (*Comparison IV*) and at high (*Comparison V*) P_{O_2} level, produced significant decreases in $\dot{V}_E$ and V_D/V_T, a small reduction in $\dot{V}_A$ and f, no demonstrable change in V_D, and a slight increase in V_T. However, not only the nitrogen tension but also the density of the inhaled gas was altered; therefore no definite conclusions can be drawn regarding the immediate cause of these respiratory changes.

Although it has been known for many years that nitrogen at high pressures exerts a depressant action upon the higher centers of the brain (BEHNKE, THOMSON and MOTLEY 1935, MARSHALL 1951, BENNET and GLASS 1957), little is known about its effects on respiration and the medullary centers. LANPHIER (1955) reports that there is evidence that $\dot{V}_E$ is reduced significantly during exercise at increased ambient pressures, and tentatively ascribes some of the reduction to a respiratory depressant effect of nitrogen itself. Observations made in breath-holding experiments at raised ambient pressures indicate, on the other hand, that high pressures of nitrogen (up to 3.8 atm) have little, if any, depressant effect upon the respiratory centers (HESSER, HOLMGREN and BESKOW 1959). As will be shown below the reductions of $\dot{V}_E$ and $\dot{V}_A$ induced by increasing the inspired P_{N_2} might be attributed almost quantitatively to the increased breathing resistance. The conclusions may, therefore, be drawn that nitrogen at pressures of 3.0—4.0 atm has no or, at most, a very slight respiratory depressant effect, no matter whether the oxygen pressure is normal or elevated.

The respiratory effects of resistance due to increased gas density may be estimated as follows. At rates of flow up to 2 l/sec the total nonelastic pulmonary resistance is approximately doubled as the ambient pressure is raised to 4.0 atm (MARSHALL, LANPHIER and DUBOIS 1956). However, since the lung tissue viscous resistance constitute about 30 to 40 per cent of the nonelastic pulmonary resistance during quiet breathing (MCILROY *et al.* 1955) and presumably would not be increased by increased ambient pressure, the airway (gas-flow) resistance of the lungs at 4.0 atm should be increased approximately threefold. By using the formula presented by BRISCOE and DUBOIS (1958) we

have calculated that, at sea level pressure, the average airway resistance in our subjects amounted to 1.2 cm H_2O/l/sec. In the flow range in question (< 0.5 l/sec) the resistance of the external breathing system increased by about 2.5 cm H_2O/l/sec as the ambient pressure was raised to 4.0 atm (see Methods). Hence, at this pressure the total increase in breathing resistance was about 5 cm H_2O/l/sec. Added resistance to breathing causes respiratory changes which are qualitatively similar to those observed in the present experiments, *i. e.* a reduction of $\dot{V}_E$ and f, and an increase of V_T and alveolar P_{CO_2} (CAIN and OTIS 1949, ZECHMAN, HALL and HULL 1957). From the data published by CAIN and OTIS it can be calculated that an added resistance of 6.7 cm H_2O/l/sec brought about a 4 per cent decrease in $\dot{V}_E$, a 16 per cent decrease in f, and a 14 per cent increase in V_T, simulating our calculated average figures of *Comparison IV and V* of approximately 9, 19 and 21, respectively. The respiratory effects of increasing the inspired P_{N_2} by about 3.0 atm may, therefore, be attributed almost quantitatively to the added breathing resistance. It may then be assumed that without an external breathing system the alterations in respiration would have been less.

No data seem to have been published as to the effect of high P_{N_2} on V_D. Our results indicate that V_D remains unaltered as the inspired P_{N_2} is increased by 3.0 atm, which also suggests that changes in density of the inhaled gas have no demonstrable effect on V_D. This agrees with BARTELS *et al.* (1954), who found that breathing helium (density approximately one fifth that of air) did not alter the oxygen, carbon dioxide or nitrogen dead spaces.

As a consequence of an unaltered V_D and an increased V_T, the fraction V_D/V_T decreased significantly as the inspired P_{N_2} was raised by 3.0 atm.

Effects of Simultaneous Changes in Inspired P_{O_2}, Inspired P_{N_2}, and Gas Density. With increasing ambient pressure (breathing air) the respiratory movements became progressively slower and deeper, and, up to about 3.0 atm, $\dot{V}_E$ gradually decreased. At 4.0 atm V_D/V_T was decreased, and, as a consequence of a lowered $\dot{V}_D$, the effective alveolar ventilation somewhat increased in spite of a reduced $\dot{V}_E$. At the same time there were no demonstrable changes in R, V_D, or in expiratory reserve volume (*Comparison I*).

A decrease in respiratory rate at increased ambient pressures has also been reported in the earlier literature (see BEAN 1945). Although LAMBERTSEN *et al.* (1953 c) found no significant changes in f, V_T and $\dot{V}_E$ as the ambient pressure was raised to 3.5 atm, the average changes in their six subjects were similar to ours. Inter-individual differences in such a small group of subjects may account for the fact that the changes observed were not significant. In our experiments the existence of large inter-individual variations in the respiratory responses to raised barometric pressures has been clearly demonstrated. Nevertheless, at 4.0 atm our group of subjects showed a statistically significant decrease in f and $\dot{V}_E$, and a significant increase in V_T.

The independent effects of changes in inspired oxygen and nitrogen pressures, discussed previously, indicate that the gradual decrease in f and gradual increase in V_T with increasing ambient pressure were to a greater part caused by the successive increase in air density and hence in breathing resistance, and to a lesser part by the concomitant increase in inspired P_{O_2}.

Although our results show that, with increasing ambient pressure, $\dot{V}_E$ decreased gradually up to 3.0 atm only to increase again with a further increase in pressure to 4.0 atm (Fig. 1, *A* and *D*), the number of observations is too small to make this change in the relationship between ambient pressure and $\dot{V}_E$ statistically significant. However, the validity of such a change is substantiated by the following reasoning. The influence of increased O_2 tension on the respiratory activity is probably dependent on the magnitudes of two opposing effects, *viz.* an increase in activity of the respiratory center due to local accumulation of CO_2 and a reduction of chemoreflex drive (LAMBERTSEN *et al.* 1953 a, b and c, SHEPHARD 1955). This implies that in conditions where an increase in O_2 tension causes a greater reduction in chemoreflex drive than is offset by local accumulation of CO_2, the respiratory activity will decrease. On the other hand, in conditions where the O_2 tension is higher than the threshold value of the chemoreceptors, an increase in O_2 tension will cause an increase of the respiratory activity. In cats WITZLEB *et al.* (1955) found the O_2 threshold of the chemoreceptors of the carotid bodies to be about 120 mm Hg, but the existence of some chemoreceptor activity above this P_{O_2} level could not be ruled out. From their experiments on humans DEJOURS *et al.* (1957) concluded that the chemoreflex drive was abolished when the inspired oxygen was above 33 per cent. However, in breath-holding experiments we have found evidence that the O_2 threshold in humans may be as high as 350—400 mm Hg (HESSER, HOLMGREN and BESKOW 1959). With increasing ambient pressure (breathing air) the respiratory activity would then first decrease as a result of the concomitant increase in P_{O_2}, only to increase again when the alveolar P_{O_2} exceeds about 400 mm Hg, *i. e.* when the ambient pressure exceeds 3.0 atm. We have therefore concluded that the gradual reduction in $\dot{V}_E$, induced by raising the ambient pressure successively up to 3.0 atm, was caused partly by the concomitant increase in P_{O_2}, and partly by the gradual increase in breathing resistance. This last conclusion was derived from our observations concerning the respiratory effects of changes only in inspired P_{N_2}. The reduction in $\dot{V}_E$ at 4.0 atm was, on the other hand, probably caused only by the increase in breathing resistance.

The observation that the reduction of $\dot{V}_E$ at 4.0 atm was accompanied by an increase rather than a decrease of $\dot{V}_A$ is supported by the results of LAMBERTSEN *et al.* (1953 c). At 3.5 atm they found no consistent changes in the CO_2 output but a significant reduction of the alveolar P_{CO_2}, which should imply an increase of $\dot{V}_A$. From our observations on the independent effects

of changes in inspired oxygen and nitrogen pressures we have concluded that the small increase in $\dot{V}_A$ at 4.0 atm was due to the respiratory stimulating effect of high P_{O_2} being somewhat greater than the suppressing effect on respiration of increased breathing resistance.

To our knowledge no data have been published previously as to the effects of raised barometric pressures on R and V_D. Our findings that R was not measurably altered as the ambient pressure was raised to 4.0 atm suggest that no important interference with the pulmonary gas exchange occurs even when the density of the respired gases is increased to four times that at sea level. This is supported by the above-mentioned observations of LAMBERTSEN *et al.* (1953 c), but is at variance with findings reported in earlier literature that an increase in ambient pressure causes a reduction in the removal of CO_2 by influencing diffusion resistance and gaseous exchange in the lungs (BEAN 1945).

The observations that V_D/V_T was decreased at 4.0 atm whereas V_D remained unaltered can apparently be assigned to the combined effects of high P_{O_2} and increased gas density since both factors caused an increase in V_T and no measurable change in V_D.

Summary

The respiratory effects of raised barometric pressures were studied on eight healthy subjects at rest in a recompression chamber. By comparing data obtained on air, 100 % O_2, and 5 % O_2 in N_2 at various ambient pressures the independent effects of changes in oxygen and nitrogen pressures were studied. The following observations were made.

1. With increasing ambient pressure (up to 4.0 atm) respiration became progressively slower and deeper. The reduction in respiratory rate and the increase in tidal volume were caused to a greater part by the increase in gas density and hence in breathing resistance, and to a lesser part by the concomitant increase in oxygen tension.

2. On increasing the ambient pressure from 1.0 to 4.0 atm the respiratory minute volume gradually decreased to 3.0 atm, and then increased slightly to 4.0 atm. Evidence is presented that the gradual decrease was caused partly by the increasing breathing resistance, and partly by the removal of chemoreflex drives due to the concomitant increase in oxygen tension. The reduction in minute volume at 4.0 atm was probably entirely due to the increased breathing resistance.

3. At 4.0 atm the calculated effective alveolar ventilation was somewhat increased. It was concluded that this increase was caused by the respiratory stimulating effect of high oxygen tension being greater than the suppressing effect on respiration of increased breathing resistance.

4. Functional dead space volume showed no consistent difference in any of the conditions studied, which confirms the findings of other investigators that the volume of the dead space is independent of the inspired oxygen tension and the density of the inhaled gas.

5. At 4.0 atm the dead space/tidal volume ratio was decreased as a consequence of an unaltered dead space volume and an increased tidal volume.

6. Raising the ambient pressure to 4.0 atm caused no consistent change in the respiratory exchange ratio, which suggests that no important interference with the pulmonary gas exchange occurs even when the density of the inhaled gases is increased four times.

7. Expiratory reserve volume showed no significant change in any of the conditions studied, which suggests that the lung volume at the mid-respiratory level increased with raising ambient pressure since the tidal volume then increased.

8. Evidence is presented that nitrogen at pressures of 3.0—4.0 atm exerts little, if any, depressant action on respiration, no matter whether the oxygen tension is normal or elevated.

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Studies on the Possibilities of Improving the Nutritive Value of Swedish Wheat Bread

I. The effect of lysine supplementation

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Abstract

Ericson, L.-E. and C. Ovenfors. Studies on the possibilities of improving the nutritive value of Swedish wheat bread. I. The effect of lysine supplementation. *Acta physiol. scand.* 1959. *47.* 44—51. — The growth rate of weanling albino rats was used as an index of the extent of improvement in the nutritive value of wheat bread that is obtainable by lysine supplementation. It was found that the addition of 0.5 % L-lysine to a basal diet prepared from 90 % air dried wheat bread (containing 3.3 % roller dried skim milk) and ample quantities of vitamins, minerals and essential fatty acids, doubled the growth rate of the rats and caused a gain in body protein. However, this growth rate was still only about half that obtained with a stock diet or a diet containing egg protein. Possible explanations for this relatively small effect of lysine supplementation and for the differences between our results and those of other authors have been discussed.

In 1914 Osborne and Mendel found that the addition of lysine to a diet derived from the wheat protein gliadin increased the growth rate of weanling rats (Osborne and Mendel 1914). From this and other observations (Osborne and Mendel 1919) they concluded that lysine is an amino acid that can not be synthesized by the rat at a rate compatible with normal growth, and that

wheat does not supply enough lysine to satisfy the requirement of the growing rat.

Following the original findings of OSBORNE and MENDEL, the improvement of the nutritive value by the addition of lysine to diets in which the major part of the nitrogen has been supplied as wheat protein has been observed by many research workers. Not only rats but also dogs, chickens, turkeys and protozoa have served as experimental animals. It is of special significance that beneficial effects of lysine supplementation have also been observed in experiments with both adults and infants.

A significant portion of the total protein consumption is derived from wheat in many countries of the world. In Europe the wheat consumption is highest in some of the southern countries where wheat sometimes supplies as much as 50 per cent of the total protein intake. The wheat consumption in Scandinavia is lower. In Sweden, for instance, approximately 20 per cent of the total protein intake comes from wheat. The possibility of improving the nutritive value of this important commodity has naturally attracted a great deal of interest. Extensive research is currently being carried out both in the USA and in the United Kingdom in order to evaluate the extent of improvement obtainable by lysine fortification and to develop processes suitable for large scale production of this amino acid.

In the present paper some observations are described concerning the growth promoting effect of lysine supplementation of Swedish wheat bread.

Experimental

Two experiments using two different makes of bread were carried out. Male weanling rats were used in both experiments and the animals were fed *ad libitum*. The rats were housed individually in glass containers (with a diameter of 20 cm) containing wood shavings.

The diets. All bread diets contained 90 % wheat bread, 3 % of a salt mixture, 4.5 % soya bean oil, 0.2 % cod liver oil and a vitamin mixture.

The salt mixture was that described by HEGSTED *et al.* (1941). The vitamin mixture (HARPER *et al.* 1953) provided the following quantities of vitamins in mg per 100 g of ration: thiamine · HCl 0.5, riboflavin 0.5, niacin 1.0, calcium pantothenate 2.0, pyridoxine 0.25, biotin 0.01, pteroylglutamic acid 0.02, cyanocobalamin 0.002, inositol 10, menadione 0.5, and choline chloride 150. The cod liver oil supplied approximatively 150 I. E. vitamin A and 15 I. E. vitamin D per 100 g of ration.

The bread used in the first experiment ("Formbröd") had been baked from a dough with the following composition:

water	1 litre
wheat flour (70 % extraction)	1.8 kg
lard	40 g
roller dried skim milk (< 1 % fat)	100 g
sugar	20 g
salt	10 g
malt extract	20 g
yeast	70 g per litre dough.

The dough for the bread used in the second experiment ("Långfranska") had a similar formula:

water	1.0 litre
wheat flour (70 % extraction)	1.8 kg
lard	28 g
roller dried skim milk (< 1 % fat)	100 g
sugar	5 g
salt	30 g
malt flour	5 g
yeast	65 g per litre dough.

The bread was cut in cubes approximatively 2 × 2 × 2 cm and dried over night in a stream of air having a temperature of about 26° C. After this treatment it contained 92 % dry matter and was ground in an Electrolux "Assistent" bread mill.

The nitrogen content of the nonsupplemented bread diets was 1.85 %.

The lysine content of the nonsupplemented bread diets was 2.4 g per 16 g N (corresponding to 0.30 % of the dry weight) for both types of bread.

In the first experiment, three different diets were fed to three groups of rats, each group containing (originally) six rats. Group 1 received the bread diet + 0.5 % glycine, group 2 the bread diet + 0.5 % L-lysine and group 3 a stock diet containing 21 % protein (N×6.25) of both animal and vegetable origin.

In the second experiment six groups of rats with eight rats in each group were fed six different diets. The first group received the bread diet + 0.6 % glycine, the second group the bread diet + 0.4 % glycine + 0.2 % L-lysine · HCl, the third group the bread diet + 0.2 % glycine + 0.4 % L-lysine · HCl, and the fourth group the bread diet + 0.6 % L-lysine · HCl. The fifth and the sixth groups were given diets in which all the protein was supplied as roller dried skim milk and whole spray dried egg respectively. Diet number five contained 32.5 % dried milk and diet number six 27 % whole dried egg, which gave these two diets the same nitrogen content as the bread diets. The same salt and vitamin mixtures and the same amounts of cod liver oil and soya bean oil as was used for the bread diets were added to the milk and the egg diets. To the egg diet, however, twice as much biotin was added as to the other diets. The milk and the egg diets were adjusted to 100 % by the addition of wheat starch.

Analytical procedures. Nitrogen determinations were made according to a Kjeldahl method using mercuric oxide as the catalyst (PERRIN 1953). The ammonia was collected in boric acid and titrated with 0.01 N hydrochloric acid. Samples were run in triplicates.

Dry weight determinations were carried out by heating the sample at 104° C for 20 h.

Total lipid content was estimated by a 6 h extraction of dried and ground samples with ether and alcohol in a Soxhlet apparatus.

Lysine was determined microbiologically using *Leuconostoc mesenteroides* P-60 according to the method of STEEL *et al.* (1949).

Results

Fig. 1 shows the growth curves over a period of five weeks for the three groups of rats used in the first experiment. The group receiving the bread diet without lysine is designated B, the group given the lysine supplemented bread diet L, and the group given the stock diet S. As can be seen from the figure, a considerable increase in the growth rate was obtained as the result of the addition of 0.5 % L-lysine to the bread diet. However, the growth of

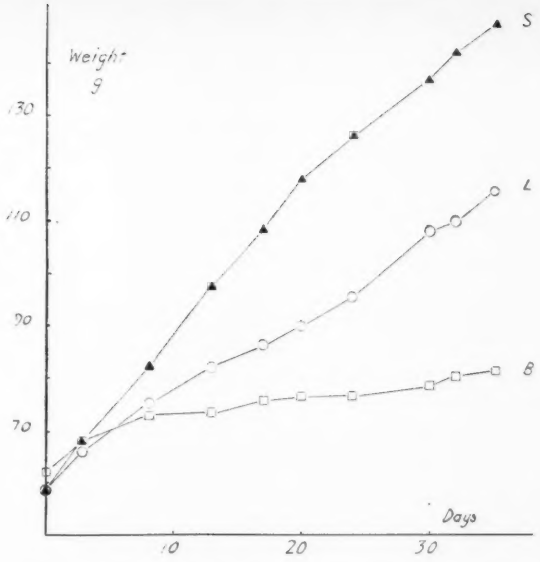

Fig. 1. Growth curves for the rats used in the first experiment. B = bread diet, L = bread diet + 0.5 % L-lysine and S = stock diet. The standard error of the mean (referring to the average final weight) was: group B ± 7, group L ± 6, group S ± 9.

the group receiving lysine was not comparable to the growth of the rats receiving the stock diet.

It should be pointed out that a respiratory infection killed some of the rats used in this experiment. Only four of the six rats in group B survived the entire experimental period, whereas five of the six rats survived in groups L and S. The results must therefore be considered only as indicative as is also reflected by the large "standard error of the mean" (see text to fig. 1).

In the second experiment six groups of rats with eight rats in each group were given the basal bread diet (B), the basal diet supplemented with 0.2, 0.4, and 0.6 % L-lysine · HCl (L02, L04 and L06), a dried milk diet (M) and an egg diet (E). Fig. 2 shows the rate of growth for these groups during the nearly five weeks of the experiment. A significant increase in the growth rate was obtained by the addition of as little as 0.2 % L-lysine · HCl (0.16 % L-lysine) to the basal bread diet. Supplementation of the bread with 0.4 or 0.6 % L-lysine · HCl caused a further increase in the growth rate; the rate of growth of the group receiving 0.6 % L-lysine · HCl (0.48 % L-lysine) was approximately twice as great as that of the group given the basal bread diet. However, the difference is slight between the growth rate of the group L02 on the one hand and that of either of the groups L04 or L06 on the other

Table I. Average final weight and total protein content for three rats from each group of the second experiment

Group	Weight	Protein content	Group	Weight	Protein content
	g	g		g	g
B	115	23.9	LO6.....	153	31.7
LO2.....	146	30.4	M.....	169	38.7
LO4.....	151	--	E	205	46.0

hand. The increase in the biological value of the bread protein that is obtained on addition of more than 0.2 % L-lysine · HCl is therefore so small, that it hardly justifies the increased cost that would arise from the use of a higher concentration of lysine.

None of the groups of rats receiving the bread diet supplemented with lysine grew as fast as the groups receiving the diets based on dried milk (M) or whole egg (E). The difference between the growth rate of the group given the whole egg diet (E) and that of group L06 is striking.

It is known that growth is not necessarily equivalent to a gain in body nitrogen (ALLISON 1949). An attempt was therefore made to estimate the amount of nitrogen and fat in the rats from the second experiment. Three rats from each group were killed with chloroform, frozen and homogenized in a Waring blendor together with a known amount of water. The homogenate was analyzed for percentage dry matter, nitrogen, fat and lysine as described under Experimental. No significant difference in the concentrations of nitrogen was observed between the various groups of rats. This is shown in Table I, where the final total protein content (N × 6.25) has been calculated.

It is thus evident that the observed gain in weight obtained on supplementation of a bread diet with lysine represents a gain in body protein.

Also the differences in fat content and in the content of lysine in g per 16 g N were found to be small and do not justify further comment.

Discussion

ROSENBERG and ROHDENBURG (1952) and ROSENBERG, ROHDENBURG and BALDINI (1954) have obtained results which indicate that supplementation of bread containing 3 % of nonfat milk solids with lysine gives a diet that stimulates the same rate of growth in weanling rats when fed at a 12 % protein level, as does a stock diet containing 21.5 % protein of both animal and vegetable origin. Also other authors (cf. FLODIN 1953; FELDBERG and HETZEL 1958) have expressed the opinion that addition of lysine to wheat bread gives a product having a biological value similar to that of animal protein. Our results

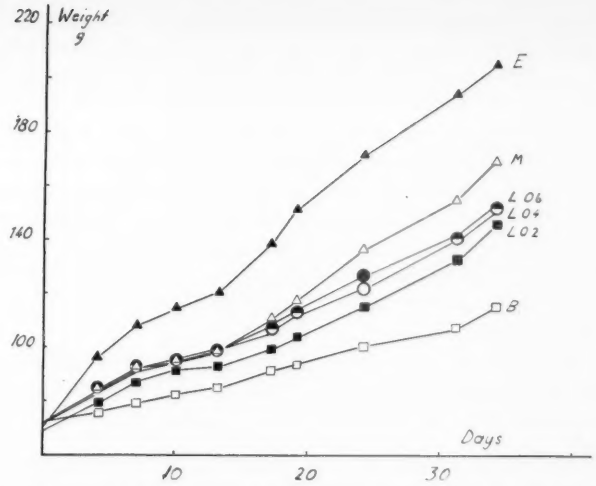

Fig. 2. Growth curves for the rats used in the second experiment. B = bread diet; L02, L04 and L06 = bread diet + 0.2, 0.4 and 0.6 % L-lysine·HCl; M = milk diet and E = egg diet. The standard error of the mean (referring to the average final weight) was: group B ± 5, group L02 ± 3, L04 ± 6, L06 ± 3, M ± 5 and E ± 5.

do not agree with such a conclusion. In our experiments the rats fed either a stock diet containing 21 % protein (N × 6.25) or a diet in which all the protein (12 %) was derived from spray dried whole egg, grew about twice as fast as the rats receiving the basal bread diet supplemented with up to 0.5 % L-lysine and containing about 11 % protein (N × 5.70).

The difference between the growth on the stock diet employed in the first experiment and on the lysine supplemented bread diet can probably be explained on the basis of the difference in protein content of the two diets. However, this explanation can not be used to interpret the discrepancy between the growth rates of the groups receiving the egg diet and the lysine supplemented bread diet (second experiment), as the two diets were fed at the same nitrogen level. The egg diet is likely to have a considerably higher fat content than the bread diet (the spray dried whole egg used contained approximately 40 % ether-alcohol soluble material). It is known that fat has a sparing action on protein but it seems doubtful that this could explain differences of the magnitude encountered here.

It seems more likely that the reason for the slower growth rate of the rats receiving the lysine supplemented diet as compared to the rats given the egg diet is due to the fact that the biological value of the bread protein — in spite of the lysine supplementation — is inferior to that of the egg protein. Support for this assumption is provided by the recent observations of HUTCHINSON,

MORAN and PACE (1958) and BENDER (1958) who have obtained an increase in the biological value of lysine supplemented bread protein on addition of threonine or of threonine and methionine. ROSENBERG, ROHDENBURG and BALDINI (1954), on the other hand, obtained no increase in the growth rate or protein efficiency when threonine, methionine or valine was added to a bread diet already supplemented with lysine.

Different varieties of wheat can have rather different nitrogen contents and amino acid compositions (cf. LYMAN, KUIKEN and HALE 1956, HARVEY 1956) and the variations in the nitrogen contents and the amino acid spectra of flour of different extractions must be expected to be even more pronounced. This might be a factor that should be considered when comparing the results of different research groups.

Whatever the reason is for the discrepancies between our results and those of other authors, it is evident that more work is required before these differences can be satisfactorily explained.

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Acta physiol. scand. 1959. 47. 52—55

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Transaminase Activity in Normal and Denervated Skeletal Muscle in Rabbits

By

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Received 21 February 1959

Abstract

Flodmark, S., G. Jungner and I. Petersén. Transaminase activity in normal and denervated skeletal muscle in rabbits. *Acta physiol. scand.* 1959. *47.* 52—55. — A study has been made of the transaminase activity in normal and inactivated muscles of rabbits. Specimens were taken from differently functioning muscles of 30 adult animals, and from muscle that had been denervated for different lengths of time, and the transaminase activity (GOT) per mg of dry tissue was determined according to Ordell's method. It was found that the activity was significantly higher in the rapidly functioning tibialis anterior muscle than in the biceps femoralis muscle. It was also significantly greater in normal than in denervated muscle.

It is a common clinical observation that the quantity of the muscular mass undergoes changes during diseases which primarily or secondarily affect the muscles. However, it has been hard to determine the nature of these changes biochemically. The transaminases, the enzymes which catalyze the reversible reaction amino acid—keto acid, one of the series of reactions of prime importance to the connection between protein and carbohydrate metabolism, have been given a great deal of attention during recent years by both chemists

Table I. Transaminase Activity in Normal Skeletal Muscle

	No. of Determinations	Arithmetical Mean of Transaminase Activity, Units/mg Dry Tissue	Range Obtained
M. biceps femoralis	48	59.3	22—201
M. tibialis anterior	46	135.9	34—320

The difference between 59.3 and 135.9 is statistically significant ($P < 0.01$).

and clinicians (KARMEN, WRÓBLEWSKI and LA DUE 1955). As both the striated and cardiac muscle contain a large amount of transaminase, it has been studied if it is possible to establish whether a muscle is injured from the amount of transaminase in the blood *e. g.* in cardiac infarction (LA DUE, WRÓBLEWSKI and KARMEN 1954, STEINBERG and OSTROW 1955). Some authors, studying cases of cardiac infarction, have observed a relationship between a muscular injury and the amount of serum transaminase (CHINSKY and SHERRY 1957). Other authors have used animals to study the degree of transaminase loss in the heart muscle after ischemia of varying duration (JENNINGS, KALTENBACH and SMETTERS 1957). The relation between the way a muscle functions and the amount of transaminase it contains, however, has only been analyzed by a few authors. BARBIERI reported in 1957 that the GOT (glutamic-oxalacetic transaminase) activity in the beef heart is lower in the muscles of the right auricle than in those of the left ventricle. The same author (1955, unpublished study) said that he had demonstrated that the amount of transaminase that different striped muscles contain is proportional to the amount of work they do.

The aim of the present study was to get normal values for the transaminase activity in different muscles of the rabbit, and to begin a study of the transaminase content in denervated muscles of the same animals.

Methods

Our experiments were done with 30 adult rabbits, weighing between 3 and 5 kg. Twenty-one were used only to get normal values for M. biceps femoris and M. tibialis anterior. Nine of those were also used to see whether the transaminase activity of the muscles changed after they were denervated. The sciatic nerve was severed on a level with the upper third of the thigh and after varying lengths of time pieces of muscle were removed from the front of the corresponding lower leg.

Biochemical Method. After homogenization of the muscular specimen, the GOT (glutamic-oxalacetic transaminase) activity was determined with ORDELL's (1956) modification of the method originally elaborated by KARMEN *et al.* (1955). The values obtained were corrected for the water in the muscle, and the activity was expressed in GOT units per mg of dry tissue.

Table II. Comparison Between Transaminase Activity in Normal and Denervated Skeletal Muscle

	No. of Determinations	Arithmetical Mean of Transaminase Activity, Units/mg Dry Tissue	Range Obtained
Normal M. tibialis anterior	46	135.9	34—320
Denervated M. tibialis anterior..	15	75.8	21—167

The difference between 135.9 and 75.8 is statistically significant ($P < 0.01$).

Results

The results are seen from Table I—III.

In 3 of the 9 animals whose sciatic nerves were severed the corresponding muscles of the lower leg were found to be distinctly atrophied when the specimens were removed. The transaminase values in these cases were 64, 21 and 181 units.

Discussion

As seen from Table I, there was a significant difference between the transaminase activity in the normal biceps femoris and tibialis anterior muscles. It is possible that there is a connection between the amount of transaminase the muscles contain and the way they work, *e. g.* a high content in delicate, quickly moving muscles like the tibialis anterior and a low content in heavy, slowly moving muscles like the biceps femoris. However, no definite conclusions can be drawn until several parallel studies of different muscles have been done. As seen from Table II, there was also a significant difference between the transaminase activity in the normal and denervated tibial muscles. According to the results in Table III, the activity did not differ significantly at different periods of time after the denervation, but only 9 animals were used. The significant difference between the transaminase activity in normal and denervated muscle is interesting, but more research is needed before it is quite clear what it signifies. Gross atrophy was observed in the muscles of 3 of the 9 animals when specimens were taken from them. Specimens of atrophic muscle may contain more connective tissue in proportion to muscular tissue than nonatrophied muscle and consequently show a lower transaminase activity. This source of error was partly eliminated in that only muscular specimens with about the same percentage of dry tissue were included in the series.

Table III. Transaminase Activity in Denervated Tibialis Anterior Muscle at Different Intervals After the Sciatic Nerve was Severed

	No. of Determinations	Arithmetical Mean of Transaminase Activity, Units/mg Dry Tissue	Range Obtained
½—4 weeks after denervation of M. tibialis anterior	10	99.3	41—167
6—12 weeks after denervation of M. tibialis anterior	15	76.2	21—108

The difference between 99.3 and 76.2 is not statistically significant ($0.01 > P < 0.02$).

Summary

1. The transaminase activity in the biceps femoris and tibialis anterior muscles of 30 rabbits was determined.

2. The arithmetical mean of the transaminase activity expressed in units per mg of dry tissue amounted to 59.3 for the biceps femoris and 135.9 for the tibialis anterior muscle. The difference is statistically significant.

3. The sciatic nerve of 9 of those rabbits was severed and specimens of the tibialis anterior muscle removed after varying intervals, from a few days to three months later, showed an arithmetical mean of 75.8 units. This mean differed significantly from the mean of 135.9 units observed in normal tibialis anterior muscles.

4. The importance of these two observations is discussed.

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Implantation of Electrodes into the Hypothalamus of the Dog by the Diasphenoid Route

By

B. Andersson

Received 27 February 1959

Abstract

Andersson, B. Implantation of electrodes into the hypothalamus of the dog by the diasphenoid route. *Acta physiol. scand.* 1959. *47.* 56—62. — A method is described for implantation of electrodes into the hypothalamus of the dog through the sphenoid bone. The leads of the electrodes are brought out on the back of the animal. Electrodes implanted in this way may be used for several months to study effects of hypothalamic stimulation in the conscious dog.

Rather accurate placement of electrodes in the brain stem may be achieved in many species by the use of stereotaxic instruments. As pointed out by Hume and Ganong (1956), however, localization by stereotaxic coordinates based on skull planes is unsatisfactory in the mongrel dog, because of the great variability in the shape and size of the skull, and in the relation of the bony landmarks to the brain. This is one main reason why the conscious dog has rarely been used to study effects of hypothalamic stimulation. Hume and Ganong (1956) have developed an elaborate method for the accurate placement of electrodes in the hypothalamus of the dog. Their method is, how-

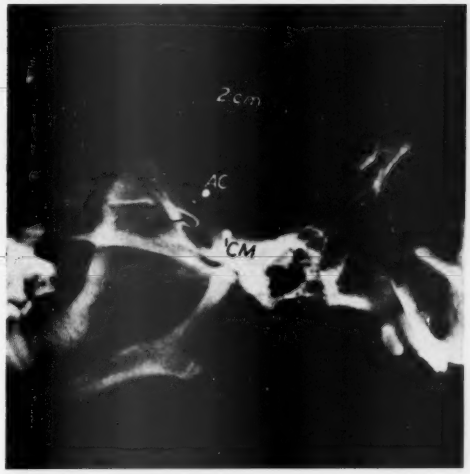

Fig. 1 A. An X-ray picture of the sagittally split head of a Samoyede dog with pieces of metal placed in the positions of the optic chiasm (C. O.), the anterior commissure (A. C.) and the mammillary body (C. M.).

ever, complicated as it involves the use of a stereotaxic instrument and the performing of ventriculogram during the operation. Electrodes implanted from the roof of the skull must further involve special care of the animals after the operation. In order to overcome these difficulties the following technique was developed.

1 B. The sagittally split cranium of the same dog demonstrating how electrodes may be implanted into the hypothalamus by the diasphenoid route by help of a perspex plate (p) fixed on to the sphenoid bone.

Orientation at the base of the skull

Studies in dead animals. The intact and sagittally split heads of dogs belonging to six different breeds (Collie, German sheepdog, Harrier, Airedale terrier, Samoyede and Dachshund) were studied on X-ray pictures. On the sagittally split heads the positions of the optic chiasm, the anterior commissure and the mammillary body had been marked with pieces of metal (Fig. 1 A). Although the hypophyseal fossa was found to be poorly developed in some of the breeds studied, the position of the pituitary could always be determined from the bony landmarks visible on X-ray pictures taken from the side. In the same manner the position of the optic chiasm, the infundibulum and the mammillary body could be determined rather accurately. On all skulls examined, the suture between the presphenoid and the postsphenoid bone was seen to run perpendicular to the ventral surface of the postsphenoid bone towards the optic chiasm.

Orientation in the experimental animals. Some days prior to the implantation of electrodes a sewing-needle was pushed through the midline of the soft palate under thiopentone anaesthesia and its tip was fixed in the sphenoid bone. The needle was broken and removed, leaving the needle tip as a landmark in the sphenoid bone. An X-ray picture was now taken of the head from the side. On this picture the tip of the sewing-needle was visible. Guided by the result of previous X-ray studies of heads of animals belonging to the same or the most similar breed, the positions of the optic chiasm, the infundibulum, the pituitary and the mammillary body were marked on the X-ray picture. The approximative position of the anterior commissure was estimated by help of known landmarks. It was now possible to determine and mark on the X-ray picture where a perspex plate, with holes for the guidance of the drill and the electrodes, had to be placed on the sphenoid bone, in order to secure that the electrodes would pass through the infundibulum and the rostral part of the pituitary and reach their goal in the hypothalamus. The distance between the tip of the sewing-needle and the estimated position of the rostral end of the perspex plate was measured.

Material

The perspex plate. When the necessary measurements were made on the X-ray picture, four holes were drilled through a perspex plate of the shape shown in Fig. 2. This plate was later to be fixed on to the sphenoid bone by help of two small stainless steel pins (Fig. 2, p) and four silver screws. Two holes were drilled on each side of the plate converging towards the midline of the base of the plate in order to make certain that the drill and the electrodes would

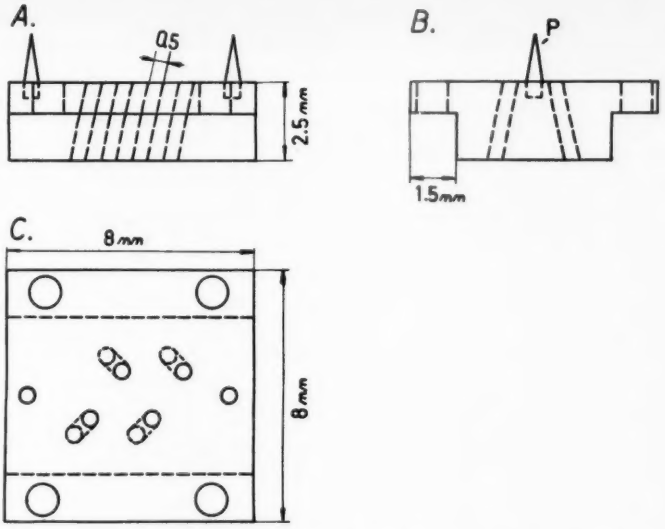

Fig. 2. A drawing showing the approximate size and the shape of the perspex plate used for guidance of the drill and the electrodes.

A: Side view.
B: Frontal view.
C: The base of the plate to be attached to the sphenoid bone.
p: Stainless steel pins used for preliminary fixation of the plate on the sphenoid bone.

pass through the inner surface of the sphenoid bone close to the sagittal plane. Fig. 3 illustrates how, by changing the site of the perspex plate and the direction of the holes through it, it was possible to reach different parts of the hypothalamus with minimal risk of hitting the circle of Willis. Each pair of electrodes was in this manner directed from one side of the nasal cavity towards the contralateral side of the hypothalamus.

The electrodes. Platinum iridium electrodes (0.3 mm in diameter) were used. A 70 cm long stainless steel wire, made of 19 separate strands (each strand 0.08 mm in diameter), was soldered on to the electrode before insulation. After insulation of the electrode and the soldered joint, a polyethylene tubing (outer diameter 0.9 mm) was thread on to the wire along its whole length and was forced over the soldered joint. The end of the polyethylene tubing, protruding slightly over the soldered joint round the base of the electrode, was filled with plastic lacquer. The electrode was then cut to the length desired and was sharpened at the free end. The insulation was scraped off to leave 0.5 mm of the free end of the electrode uninsulated.

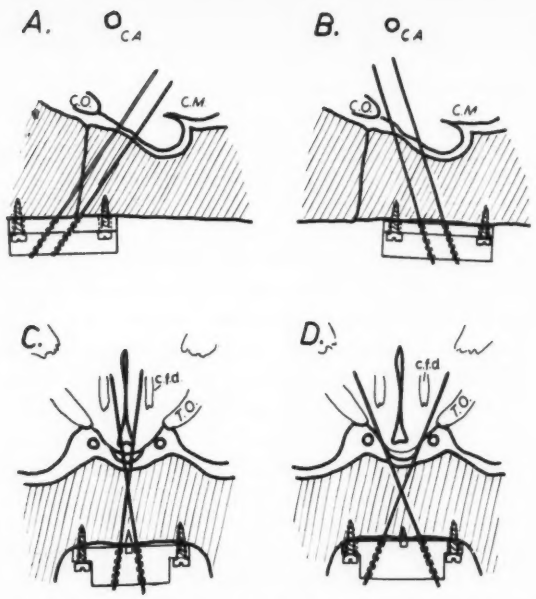

Fig. 3. Drawings to illustrate how, by changing the site of the perspex plate and the direction of the holes through it, it is possible to reach different parts of the hypothalamus with the electrodes with minimal risk of hitting the circle of Willis.

A and B: Sagittal sections through the hypothalamus and the sphenoid bone.
C and D: Transverse sections through the hypothalamus and the sphenoid bone at the level of the infundibulum.
C. A.: Anterior commissure.
C. M.: Mammillary body.
C. O.: Optic chiasm.
c. f. d.: Columna fornicis descendens.
T. O.: Tractus opticus.

Operation for implantation of the electrodes

During the operation the dog was anaesthetized with nembutal. The sphenoid bone was exposed in the manner described by ASCHNER (1912), making the tip of the sewing-needle visible. The perspex plate was preliminary fixed at the calculated distance from the needle tip by pressing the two pins of the plate (Fig. 2, p) into the midline of the bone. The plate was then definitely fixed with four silver screws. Directed by the holes in the plate, four holes (0.45 mm in diameter) were gently drilled through the sphenoid bone. The electrodes were pushed through these holes until the ends of the polyethylene

tubings were resting on the ventral surface of the perspex plate (Fig. 1 B). The whole plate and the electrode ends of the polyethylene tubings were then covered with dental cement. When the cement had hardened, a small incision was made in the skin ventral to the zygomatic process of the malar bone on each side of the head. Two cannulae with an inner diameter fitting the polyethylene tubing were from each incision run through the cheek and further under the mucous membrane of the mouth through the nasal wall of the soft palate to emerge in the nasal cavity rostral to the perspex plate. The leads were run through the cannulae out through the skin incisions. After the removal of the cannulae, the soft palate was closed with two layers of sutures. Via two incisions at the back of the head between the ears and with the help of the cannulae, the leads were now drawn subcutaneously to emerge on the animal's back between the scapulae. An indifferent electrode was fixed subcutaneously near the outlet of the electrode leads. The skin incisions were sutured and penicillium was given to prevent infection. The animal was dressed in a waistcoat covering the free ends of the electrode leads and was kept for a week on liquid food.

Stimulation experiments

During the experiments the electrode leads were let out through a window in the waistcoat and were connected to a contact fixed on to the waistcoat. The leads from the stimulator, which were fixed to a balanced suspender in the roof, were plugged into this contact. The animal was allowed to move freely in a pen during the stimulation experiments. Mainly unipolar stimulation was used.

Examples of effects obtained so far by the use of electrodes implanted by the diasphenoid route are polydipsia and hyperphagia. These effects have not previously been reported as result of hypothalamic stimulation in the dog.

Discussion

The present method does not allow as accurate placement of electrodes in the hypothalamus of the dog as does the more elaborate method of Hume and Ganong (1956). It may therefore not be recommended as a method of making accurately placed, bilateral electrocoagulations in the hypothalamus. It offers, however, several advantages as a method to study the behaviour of conscious dogs during stimulation at various sites in the hypothalamus. The method is relatively simple and electrodes can be placed within a certain area of the hypothalamus with fairly good accuracy. Stimulation can be made at four different sites in the same dog. As the electrodes and the leads are not visible and accessible to the animal it is not much disturbed by the experimental conditions.

One obvious risk attached to the method is the danger of hitting the arteries of the circle of Willis, especially if the goal for the electrodes is the most lateral part of the hypothalamus. A damage to any of these vessels is fatal. This risk can, however, be avoided by careful measurements on the X-ray picture taken prior to the operation.

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Interhippocampal Impulses

I. Origin, Course and Distribution in Cat, Rabbit and Rat

By

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Abstract

ANDERSEN, P. — Interhippocampal impulses. I. Origin, Course and Distribution in Cat, Rabbit and Rat. *Acta physiol. scand.* 1959. *47.* 63—90. — The present study is the first in a series concerned with the activation of the hippocampal fields CA1 and CA3 by interhippocampal impulses. A point-to-point connection was found between homotopic points in these fields. In both fields the stimulus thresholds were lowest in the pyramidal cell body and the apical dendritic shaft layers. This suggests electrical excitability of part of the dendritic membrane. The CA1 commissural response consisted of a surface positive/negative wave with a spike on the positive wave. The CA3 response was a diphasic spike followed by a negative wave with a spike superimposed.

In recent years considerable interest has been focussed upon the activation of cortical dendrites, but the manner in which these structures are excited has not been firmly established (for references see BREMER 1958). Most of the investigations concerning dendritic activation have been performed on the neocortex. Due to the extreme complexity of the cellular arrangement of the neocortex, it has occurred to some authors that less complex results regarding cortical activation might be obtained from the simpler organized hippo-

campal cortex (RENSHAW, FORBES and MORISON 1940, CRAGG and HAMLYN 1955, 1957; GREEN and MACHNE 1955, GREEN and ADEY 1956, VON EULER, GREEN and RICCI 1958).

A crucial point in the evaluation of evoked potentials is a detailed knowledge of the histology of the structure in question and its connections. The normal histology of the hippocampus and its neighbouring structures is well documented by the works of CAJAL (1911) and LORENTE DE NÓ (1934) (for further references see BLACKSTAD 1956). The most thorough experimental work concerning hippocampal connections is BLACKSTAD's study (1956) on the commissural afferents. The interhippocampal afferents make synaptic contact with certain, restricted parts of the dendritic tree of the pyramidal cells in the various fields of the hippocampus. These findings, taken together with the special architectonical arrangement of these pyramids, made it appear possible that an electrophysiological study of the interhippocampal impulses might yield some information regarding the rôle of the dendrites in cortical activation.

The results will be presented in four parts. The first is concerned with localization problems. The second part is an analysis of the response of the hippocampal field CA1 to interhippocampal impulses activating the neurons by way of the apical dendrites, and the third part is a similar analysis of the response of the field CA3 to commissural impulses activating the basal dendrites in this field. The fourth part presents some data regarding the properties of the ventral psalterium fibres conveying the interhippocampal impulses.

The purpose of the present investigation has been (i) to determine the exact origin of the interhippocampal impulses, (ii) to see whether a point-to-point localization does exist in this system, (iii) to investigate whether the pattern of termination observed by anatomical methods has any electrophysiological correlates, and (iv) to determine the pathways of the interhippocampal impulses.

Anatomical considerations

The hippocampus is situated between the subiculum (Fig. 1 A, sub.) and the dentate area (a. dent.)[1]. Cytoarchitectonically, the hippocampal cortex is rather simple with the cell bodies of its main element, the pyramidal neurons, gathered in a narrow band, the pyramidal layer (pyr.). The pyramidal cells send their apical dendrites towards the surface, represented by the hippocampal fissure (f. hipp.), while their basal dendrites are orientated towards the lateral ventricle (ventr. lat.) or the telo-diencephalic fissure (f. telo-di.).

The hippocampal pyramids are either small or giant ones. The region having only small pyramids was called CA1 by LORENTE DE NÓ (1934) (Fig. 1, A and C), while the region with giant pyramids was divided into the fields CA2 and CA3. In Golgi sections the field CA2 is small. It is difficult to distinguish from CA3 even in silver impregnated material, and almost impossibly so in thionin stained sections (LORENTE DE NÓ 1934).

[1] The histological terminology follows that of BLACKSTAD (1956).

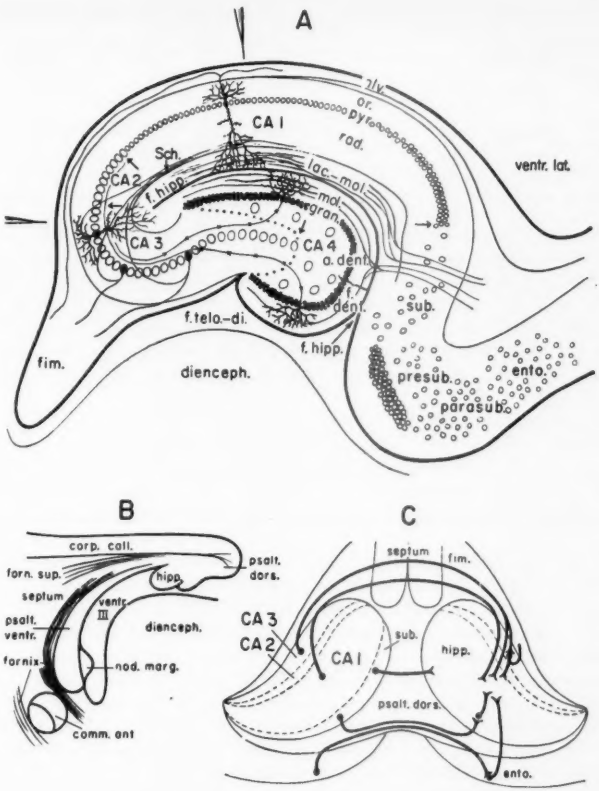

Fig. 1. Diagram of the structure and connections of the hippocampus and the dentate area. — *A*. Sagittal section from rabbit, about 3 mm from the midline. The typical recording points for the CA1 and CA3 potentials and the direction of the most used electrode tracks are indicated by the location and direction of the electrode tips. — *B*. Sagittal section in the midline. — *C*. Dorsal view of the hippocampi, the fimbriae and adjacent structures. The connections found by physiological methods are heavily outlined.

Abbreviations

a. dent.	dentate area (fascia dentata and hilus fasciae dentatae)	f. dent.	dentate fascia
		f. hipp.	hippocampal fissure
alv.	alveus	f. telo-di.	telo-diencephalic fissure
comm. ant.	anterior commissure	fim.	fimbria
corp. call.	corpus callosum	forn. sup.	fornix superior
dienceph.	diencephalon	gran.	granular layer of the dentate area
ento.	entorhinal area		
EPSP	excitatory postsynaptic potential	hil. f. dent.	hilus of the dentate fascia
		hipp.	hippocampus

lac.-mol.	stratum lacunosum-moleculare	psalt. ventr.	ventral psalterium
mol.	stratum moleculare	pyr.	pyramidal layer
nod. marg.	nodulus marginalis	rad.	stratum radiatum
or.	stratum oriens	Sch.	Schaffer collaterals
parasub.	parasubiculum	sub.	subiculum
presub.	presubiculum	ventr. lat.	lateral ventricle
psalt. dors.	dorsal psalterium	ventr. III	third ventricle

The hippocampal cortex is organized in four main layers. The most superficial is the stratum lacunosum-moleculare (lac.-mol.) containing the terminal branches of the pyramidal apical dendrites and afferent fibres from the entorhinal area (perforant path). The next layer, the stratum radiatum (rad.) is composed of the pyramidal apical dendritic shafts and various afferent fibres, also commissural ones. The pyramidal layer (pyr.) is characterized by the dense cell body packing. The stratum oriens (or.) contains the pyramidal basal dendrites and various afferent fibres of ipsi- and contralateral origin.

The CA1 pyramidal axons run towards the ventricular surface and into the alveus, which represents the white matter underlying the cortex. The CA3 pyramids send their axons more directly into the fimbria. These axons often send off collaterals which, after curving back and penetrating the pyramidal layer, course among the distal part of the CA1 apical dendrites. These collaterals (Sch.) are named after their discoverer, SCHAFFER (1892).

The most conspicuous elements of the dentate area are the granule cells, the bodies of which are assembled in an extremely dense row. Their efferent axons — the mossy fibres — penetrate the hilus of the dentate fascia (CA4) and synapse with the proximal part of the CA3 apical dendrites (LORENTE DE NÓ 1934) and probably also with those of CA2 (BLACKSTAD 1956).

Interhippocampal connections. — In the present study the term *interhemispherical* will apply to fibres or impulses crossing the midline of the brain, the term *interhippocampal* to fibres or impulses originating in the hippocampus of one side and ending in the hippocampus of the other side, whereas the term *commissural* is assigned to fibres or impulses ending in the region strictly symmetrical to the point of origin.

The hippocampus and the dentate area are connected through the ventral psalterium (ventral hippocampal commissure) with the contralateral homotopic structures (Fig. 1, B and C) (BLACKSTAD 1956).

In normal material CAJAL (1911) stated that the commissural fibres penetrate the pyramidal layer after a shorter or longer distance and end in the stratum radiatum, and LORENTE DE NÓ (1934) found that the interhippocampal fibres penetrate the pyramidal layer of CA2 and CA3 to be distributed to the stratum radiatum of all parts of the hippocampus and perhaps to the dentate area as well.

The most detailed anatomical investigation of the interhemispherical connections of the hippocampus and adjacent cortical areas has been performed by BLACKSTAD (1956) using the NAUTA technique (1950) on rats. Following contralateral hippocampal destruction terminal degeneration was found in the stratum oriens of fields CA1, CA2 and CA3 (Fig. 13), and in the stratum radiatum of CA1 and that part of CA3 which lies adjacent to the dentate area. This hippocampal degeneration was caused by lesions involving neurons sending their axons through the ventral psalterium.

Following lesions of the hippocampus and the dentate area the terminal degeneration in the opposite dentate area was extremely heavy, occupying the inner part of the molecular layer. No other parts of the dentate area showed interhemispherical terminal degeneration.

Electrophysiological studies

To the author's knowledge the only analysis of the hippocampal commissural impulses with the evoked potential technique is that of CRAGG and HAMLYN (1957) which was published during the course of the present study. In rabbits the fimbria and the hippocampus were stimulated by single shocks, and potentials recorded from the dorsal part of the contralateral hippocampus and dentate area. They concluded, in support of their anatomical findings, that the afferent commissural fibres "make synaptic contact with neurons in the field CA4 and in the anterior part of the dentate fascia, but not in the convexity of the latter". All other potentials, *e. g.* in CA1, CRAGG and HAMLYN attributed to neurons activated secondarily because these potentials were enhanced by 8/sec repetitive stimulation of the contralateral fimbria. The observations of CRAGG and HAMLYN (1957) suggested a diffuse origin of the commissural impulses in the contralateral hippocampus. Regarding the termination of commissural impulses no attempt was made to analyse the distribution of the evoked responses to different strata of the hippocampus, nor to investigate whether a point-to-point localization between the two hippocampi exists.

Material and Methods

Material. 17 adult cats (2.3—4.7 kg), 35 adult rabbits (1.8—3.4 kg) and 6 adult rats (0.3—0.4 kg) were used.

Anesthesia. A mixture of 25 per cent urethane and 1 per cent chloralose[1] was given intraperitoneally (rats and rabbits 0.75 g/kg and 40 mg/kg, cats 0.5 g/kg and 40 mg/kg, respectively). In about half of the experiments the rabbits and cats were immobilized with decamethonium bromide (0.25 mg/kg and 0.1 mg/kg respectively). The animals were connected to a vacuum-driven respirator (30 and 20 cycles/min, respectively) and given pure oxygen under positive pressure (8—10 cm and 15—20 cm water, respectively). The rats were not immobilized.

Operative procedure. After tracheotomy the dorsal calvarium was resected and the neocortex overlying the hippocampus removed by suction. In the rabbits it was also necessary to remove part of the striatum to obtain an extensive exposure of the fimbria. Neither of these procedures was seen to influence the hippocampal commissural responses. The exposed brain tissue was covered with a pool of warm mineral oil and the temperature maintained at 34° C. The room temperature remained at about 25° C.

Stimulation. Both surface and depth stimulation were carried out with bipolar electrodes consisting of two copper threads 0.2 mm diameter, lacquered together with a polar separation of 0.1 mm. The two threads were cut with a fine pair of scissors making the cut edges the only bared portions of the electrode.

Square wave stimuli were used with a pulse duration of 0.1 msec and intensities varying from 0.1 to 30 volts, usually about 5 volts. Frequency was usually 0.3/sec.

Recording. Monopolar recording was routinely used. The steel electrodes were prepared according to the method of GRUNDFEST, SENGSTAKEN, OETTINGER and GURRY (1950). The electrode tips ranged from 3 to 50 μ, most useful were those of about 20 μ. When more restricted localization was desired the bipolar stimulating electrodes, described above, were used for recording purposes. A conventional push-pull amplification system with a high impedance input stage was used. The time constant of the amplifier could be varied from 1 msec to 1,000 msec. In addition control records were taken with d. c. amplification.

[1] The chloralose was kindly supplied by E. Merck, Darmstadt.

Histology. At the end of the experiments the brains were removed and fixed in 10 per cent formalin. The electrode tracks were studied by using a modified NAUTA method (1950) for silver impregnation of paraffin sections (ANDERSEN 1956). The histological picture was compared with the potentials recorded from the various layers. The total depth of the electrode track, and small electrocoagulations at certain depths along it, served as landmarks for this comparison. The greatest error of localization was estimated to approximately 50 μ for the recording electrodes and to 100 μ for the stimulating ones. The experiments with fibre sections were controlled by the use of the same staining technique.

Results

Stimuli were delivered to points covering the whole of the exposed ventricular surface of the dorsal psalterium, the subiculum, CA1, CA2, CA3 and the fimbria (Fig. 1 C). Recordings were made from the same areas contralaterally. In addition, stimulation, as well as recording, were performed within the depth of the hippocampus by using penetrating electrodes which traversed field CA1 vertically and field CA3 approximately horizontally (indicated by the electrode tips in Fig. 1 A).

Single shock stimulation of the hippocampal or the fimbrial surface elicited two rather constant and distinct types of responses in the contralateral homotopic structures. These will be referred to as "the CA1 commissural response" (stimulating and recording electrodes located strictly symmetrically within the fields CA1 of the two sides) and "the CA3 commissural response" (the electrodes placed symmetrically in the fields CA3), both elicited by impulses traversing the ventral psalterium.

A discrete commissural response which could be attributed to the neurons of field CA2 only, was not observed. This is not surprising when it is considered that macroscopically a distinction between CA2 and CA3 is impossible. In all three species investigated CA2 is only a narrow band of tissue (Fig. 1 A and C) and it is difficult to delimit from CA3 with the usual histological methods (LORENTE DE NÓ 1934), while the transition to CA1 is sharp. For these reasons all responses ascribed to the activation of giant pyramids will be called CA3 responses even though CA2 neurons presumably contribute to a portion of these responses.

(i) *The CA1 commissural response*

Pattern of response. The potentials recorded from the surface of CA1, following single shock stimulation of the contralateral symmetrical point, consisted of a surface positive/negative diphasic potential with or without one or two spikes superimposed on the positive phase (Fig. 2 A and B). In all three species investigated the form of the potentials was similar although the time relations varied (Fig. 2 A). The spikes commonly were of either polarity or diphasic (Fig. 2 A and Fig. 7, 0 mm). In individual experiments these potentials were

usually constant and could be recorded, almost unchanged, for several hours.

The positive wave of the CA1 commissural potential had a duration of 15—30 msec (about half this duration in the rat). It was sometimes composed of two parts, an early small deflection and a prominent later one (Fig. 2 B *a* and *b*; 6 A, center record).

The latency of the early small response (measured to the first departure from the baseline) was about 4 msec when the electrodes on each side were placed at the dorsal end of the hippocampus, and about 10 msec when they were placed near the ventral temporal ends (rabbit). The corresponding values for cats were nearly double because of the longer conduction distance and slower conduction velocity of their commissural fibres (ANDERSEN 1959 c). For similar reasons the values for the rat were conversely about one-half of the rabbit values.

The negative component of the diphasic CA1 commissural response (Fig. 2 B *d*) had a duration of 30—100 msec (again about half this duration in the rat). Occasionally, in periods of high excitability of the hippocampal cortex, as in light anesthesia or following repetitive stimulation or drying of the alvear surface, the potentials were not constant from sweep to sweep. The duration of the positive and of the negative component then showed an inverse relationship, the total duration being approximately constant (Fig. 2 D). The amplitude of the two components also showed this inverse relationship. On the other hand, in periods of low excitability the surface negative wave was often lacking. The excitability level was judged from the appearance of spikes in the responses and an increased tendency to develop electrical after-discharges spontaneously or in response to electrical or mechanical stimulation.

Following stronger single shock stimulation, or repetitive stimulation, a spike, usually negative and of 1—5 msec duration, appeared superimposed on the positive wave (Fig. 2 B *c*). Still stronger stimulation would elicit a second spike (Fig. 2 A, rabbit; B). The latency of both the positive wave and the spike decreased with increasing stimulus intensities. The spike latency varied from 10 to 19 msec (rabbit) according to the electrode location. In periods of high excitability, the spike latency decreased by 1—3 msec.

Potential thresholds. When both components were present the positive and the negative wave had the same threshold (Fig. 2 B). Comparison was made with the threshold of the transcallosal potential which was recorded from a point in the neocortex in response to stimulation of the homotopic contralateral point. The transcallosal potentials were elicited between the most effective areas mapped by CURTIS (1940). The neocortex overlying the hippocampus was then removed and the CA1 commissural response was elicited by the same electrodes. Care was taken to keep the conditions of the two experiments as similar as possible with regard to temperature, moisture, handling of the cortical surfaces and the electrode pressure towards the surface. Two experiments gave the following threshold ratios for the psalterium CA1 com-

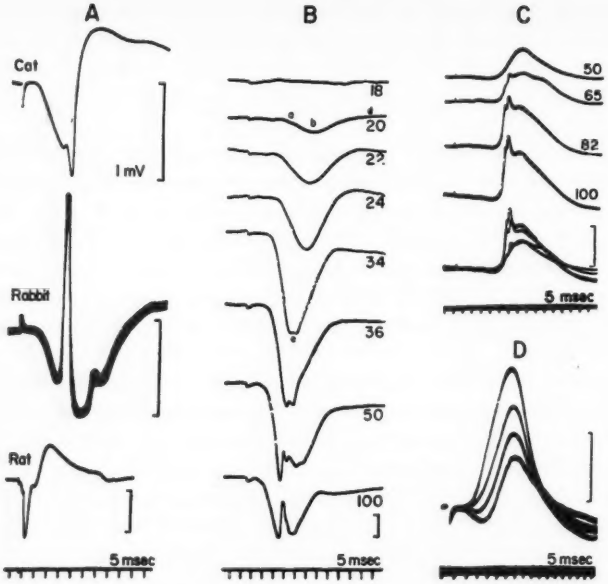

Fig. 2. Potentials evoked in CA1 following single shock stimulation of the contralateral hippocampus, the CA1 commissural response. — *A.* CA1 commissural responses from cat, rabbit and rat. Note that the spike may be of either polarity. — *B.* Effect of increasing stimulus strength (given in arbitrary units) upon the CA1 commissural response of rabbit. The different components are marked *a*, *b*, *c* and *d*. — *C.* As *B*, but recorded from the layer of the apical dendritic shafts. The last record shows the four preceding records superimposed to demonstrate that the wave duration was independent of the stimulus strength. — *D* shows the superimposition of four records obtained with 5 sec intervals in a period with increasing excitability of the hippocampal cortex (constant stimulus parameters). The negative wave starts earlier and develops greater amplitude.

In all figures except Fig. 12 the records are photographically reversed from the film. All potentials are recorded monopolarly with negativity upwards.

missural potential versus the transcallosal neocortical potential: 1/6.4 and 1/6.0. The form of the two types of potentials were strikingly similar, but the amplitude of the fully developed CA1 commissural response was greater than that of the fully developed transcallosal response (1—4 mV and 0.2—1 mV respectively). As also pointed out by CRAGG and HAMLYN (1957) the great amplitude of the CA1 response is probably due to the dense packing of the cell bodies and apical dendritic shafts in this region.

Origin of interhippocampal impulses activating CA1. In 10 experiments on 5 rabbits and 2 cats the *recording* electrode was kept fixed at the surface of CA1, and the *stimulating* electrode was moved from point to point on the contralateral hippocampus (Fig. 3). The greatest responses could be obtained by stimulating the

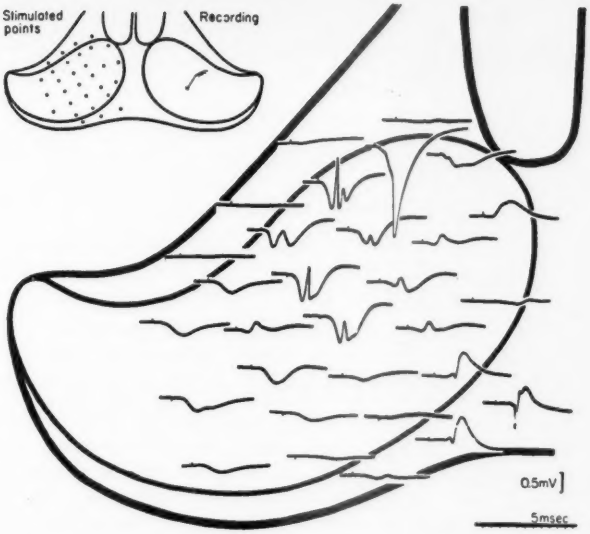

Fig. 3. Origin of interhippocampal impulses as recorded by a surface electrode in CA1 of rabbit. — The recording electrode was kept fixed at one point in the right hippocampus, the stimulating electrode was placed at various sites on the left hippocampus, fimbria and dorsal psalterium. The records obtained from the recording electrode are transferred to the corresponding contralateral stimulated points (see inset).

contralateral symmetrical point and a wedge-shaped area towards the fimbria, as well as a narrow area of the hippocampus along the border to the fimbria. The latter corresponds to the field CA3. Fig. 3 also shows that stimulation of the fimbria in this experiment did not yield contralateral CA1 potentials. Stimulation of the subiculum and the adjacent dorsal psalterium (or more likely the underlying areas, the presubiculum and the parasubiculum) elicited a potential in the opposite CA1 which was completely different from the CA1 commissural response. Usually it consisted of a surface negative wave with a positive spike superimposed (Fig. 3, lower right corner). The spike had a longer latency than the spike elicited by contralateral CA1 stimulation.

Constant intensity stimulation through a microelectrode vertically penetrating the contralateral CA1 pyramidal layer (22 experiments in 7 rabbits and 3 cats) produced the largest CA1 potentials when the electrode tip was situated within the layers of the pyramidal cell bodies and the proximal part of their apical dendrites (Fig. 4 A and C, upper halves). Similar results were obtained from stimulation of the cell body layers of CA3 (Fig. 4 C, lower half) and of CA4 (Fig. 4 A, lower half). No special effect could be ascribed to the

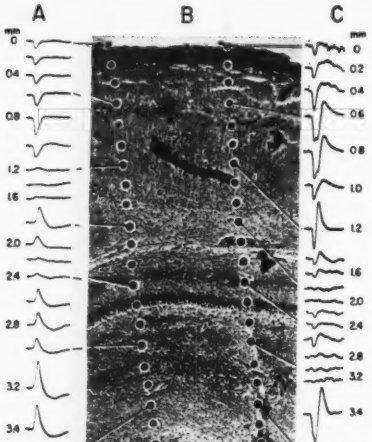

Fig. 4. Records obtained from a surface electrode in CA1 following stimulation at successive depths of the contralateral hippocampus in rabbit. — The stimulating electrode tracks are approximately parallell to the apical dendritic shafts of the CA1 pyramidal cells, starting from a point placed strictly symmetrical to the corresponding recording electrode. — *A* and *C* show the records obtained in two different experiments in two rabbits. — *B* is a photomicrograph showing the electrode track and the stimulated points of the experiment demonstrated in column *C*. The stimulated points referring to column *A* are transferred from the corresponding points in another section. — *D* is a photomicrograph of a sagittal section of the hippocampus in which is outlined the area shown enlarged in *B*.

stimulation of the thinnest branches of the CA1 apical dendrites, or of dentate granule cells. Within CA1 direct measurements showed the lowest stimulus thresholds in the pyramidal layer and in the layer of the apical dendritic shafts. These findings suggest that in addition to the cell bodies, the thicker part of the CA1 apical dendrites are also electrically excitable. On the other hand it seems to be more difficult to excite the fibres of the alveus and the fimbria than the pyramidal cell bodies or their proximal dendrites.

It is of interest that stimulation of CA3 and also of CA4 was particularly effective in evoking contralateral CA1 responses. When elicited by contralateral CA3 and CA4 stimulation, the CA1 response differed, however, from the CA1 commissural response, by exhibiting an earlier and greater surface negative phase (Fig. 4 A, lower half), especially in periods of high excitability of the hippocampus. In some experiments a pure negative wave could be elicited in CA1 by contralateral CA3 stimulation (Fig. 5). This potential (*a*)

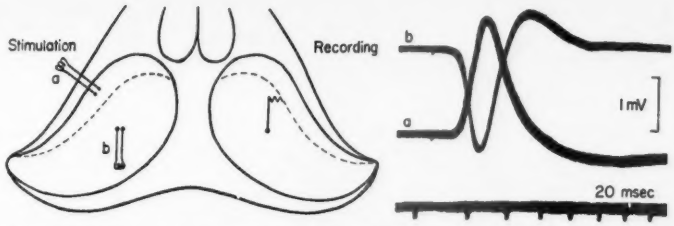

Fig. 5. Potentials obtained from a fixed surface electrode within CA1 following stimulation of the contralateral CA1 (*b*) and CA3 field (*a*) in rabbit. The cortex in this experiment showed signs of periodically increased excitability.

had the same latency as the usual CA1 commissural response (*b*) and was therefore probably directly activated, and not due to impulses relayed through the Schaffer collaterals.

Provided that there was a symmetrical electrode placement, the pyramidal cell layer of all parts of CA1 was capable of producing the described contralateral CA1 commissural potential.

Distribution of the CA1 interhippocampal potentials. In the preceding experiments the recording electrode was kept fixed within the field CA1 while the stimulating electrode was moved. In the subsequent experiments the *stimulating* electrode was kept at a fixed point in CA1 and the *recording* electrode was moved (22 experiments in 7 rabbits and 3 cats). The spatial surface distribution of the potentials evoked in the hippocampus by contralateral CA1 stimulation is shown in Fig. 6. Responses were obtained from a circumscribed part of CA1 symmetrically oriented in relation to the stimulating electrode in both the dorsal (A) and the temporal (B) part of CA1. The responsive area was greater than that of the comparable transcallosal potential evoked and recorded by the same technique (approximately 5 × 3 mm versus 2 × 2 mm). Bipolar recording made the responsive area somewhat smaller when compared with monopolar recording but the point of maximal response remained the same. Within the responsive region small differences in the latency of the surface positive wave were noted, this being shortest at the center and longer at the periphery. The latter was especially evident temporally.

The observations reported in this paragraph have been obtained in 44 out of 58 experiments (18 rabbits and 7 cats). Following contralateral CA1 surface stimulation and recording from successive depths, vertical to the CA1 surface, results were obtained as illustrated in Fig. 7. Column *A* shows the responses recorded from the different points as indicated in column *B*. The surface record presented a positive wave with a positive/negative diphasic spike superimposed, followed by a negative wave. The earliest part of the positive wave kept its polarity, but was reduced in amplitude to a minimum 1.0—1.2 mm

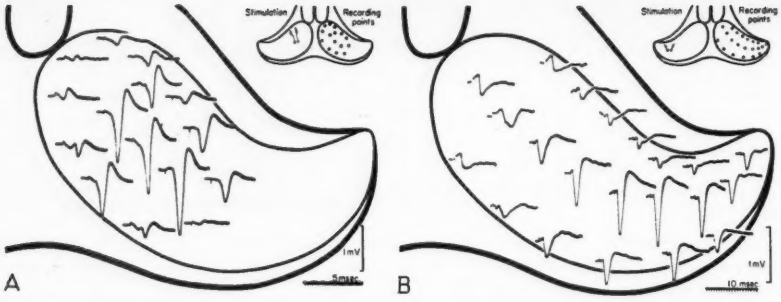

Fig. 6. Distribution of potentials in the hippocampus following contralateral CA1 surface stimulation. — Stimulation of the dorsal (*A*) and the temporal (*B*) part of CA1.

below the surface. Below the hippocampal fissure little was seen of this component (the positivity there had a longer latency). The later part of the surface positive wave reversed its polarity between 0.8 and 1.0 mm, remaining negative from 1.0 to 1.4 mm and again turning positive below 1.6 mm, which corresponds approximately to the hippocampal fissure. This positive wave rapidly vanished in the dentate area.

The surface negative wave was gradually diminished and was extinguished at a depth of 1.0 mm, corresponding to the turn-over point of the later part of the surface positive wave. Further down in the electrode track no clear sign could be seen corresponding to the surface negative wave.

The diphasic surface spike became purely negative at 0.4 mm, corresponding approximately to the pyramidal layer. Further down it remained negative till 1.2 mm which refers to the most superficial (alveus-far) part of the apical dendritic shafts. From 1.4 mm on the spike again turned diphasic, positive/negative, and at 1.8 mm (corresponding approximately to the hippocampal fissure) the spike was purely positive.

Following contralateral CA1 stimulation no specific potential was usually seen within the dentate area.

In conclusion, the first part of the surface positive potential remained positive throughout the CA1 cortex, whereas the later part of this wave reversed its polarity in the layer of the apical dendritic shafts (in most experiments it was recorded as a negative wave in the entire stratum radiatum). The surface negative wave was only recorded in the basal strata of the cortex and with the greatest amplitude near the alveus. The spike was purely negative in the pyramidal cell body layer and the area of their apical dendritic shafts, whereas it showed diphasicity (positive/negative) in the basal and in the most superficial strata of the CA1 cortex, and was purely positive in a small zone of the dentate area lying adjacent to the hippocampal fissure.

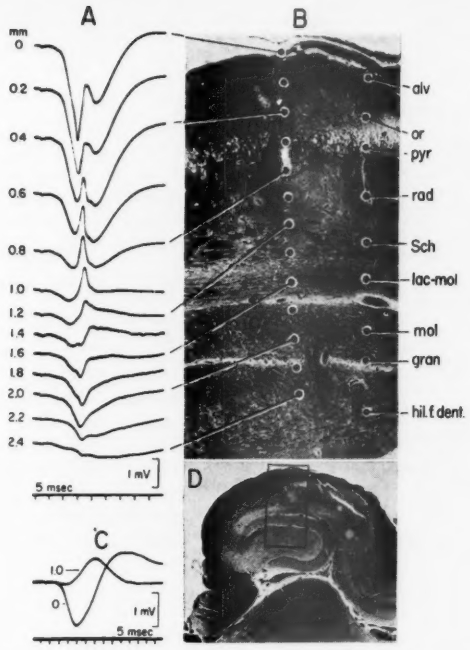

Fig. 7. Termination of interhippocampal impulses from CA1. — *A* shows responses obtained from successive depths by a recording electrode penetrating the CA1 cortex parallel to the apical dendritic shafts (rabbit). Symmetrical surface stimulation. — *B*, photomicrograph with indication of the points from which the records in column *A* were obtained. — *D* shows a sagittal section of the hippocampus with the area enlarged in *B* shown in outline. — In *C*, one record obtained from the CA1 surface following contralateral CA1 stimulation is superimposed upon one record obtained from 1.0 mm below the alvear surface (in the layer of the apical dendritic shafts).

In Fig. 7 C are shown two records from another experiment of the same type. One record obtained from 1.0 mm below the alveus (in the region of the apical dendritic shafts) is superimposed upon a surface record. The polarity reversal of the later part of the surface positive wave is clearly seen, also the small remaining positivity of the first part of this wave is shown. The surface negative wave has no correlate in the depth record.

The problem of spike latency differences in the different layers of the hippocampus and the interpretation of the various elements of the CA1 commissural potentials recorded with surface and depth electrodes is dealt with in a subsequent communication (ANDERSEN 1959 a).

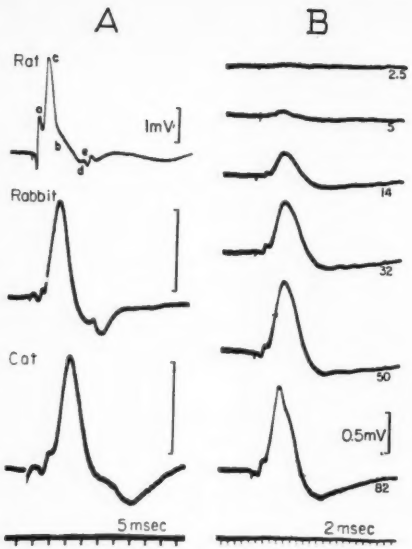

Fig. 8. Responses obtained from the surface of CA3 following single shock stimulation of the symmetrical point in the contralateral hippocampus, the CA3 commissural response. — *A*. Responses from rat, rabbit and cat. In the rat record the different components are marked *a*, *b*, *c*, *d*, and *e*. — *B*. Effect of increasing stimulus strength, given in arbitrary units (rabbit).

(ii) *The CA3 commissural response*

Pattern of response. When the stimulating electrode was placed on the hippocampus close to the fimbria (in the field CA3), and the recording electrode on the homotopic point of the other side, a quite different response from the CA1 commissural potential was obtained.

Fig. 8 A shows the CA3 commissural potential in rat, rabbit and cat. The response consisted of an early positive/negative diphasic spike (*a*) followed by a negative wave (*b*) and subsequently a positive one (*d*). The stimulus thresholds for the diphasic spike and the negative wave were the same (Fig. 8 *B*) and their amplitudes increased in a proportional manner with increasing stimulus intensities. Stronger stimuli produced a negative spike (Fig. 8 A*c* and B, 82) superimposed upon the negative wave, and sometimes one or two small spikes would develop on the following positive wave (*e*).

The duration of the negative phase of the diphasic spike was 1—2 msec in rats, 2—3 msec in rabbits and 2—4 msec in cats. These values were measured from records obtained from recording sites on the hippocampus close to the fimbria, or from the fimbria itself, about 3 mm from the midline in rats, and about 6 mm for the rabbits and cats. As the electrode was placed more laterally the duration increased while the amplitude decreased. With small electrode tips (3—5 μ), the amplitude could be as high as 1.5—2 mV (Fig. 8 A, rat).

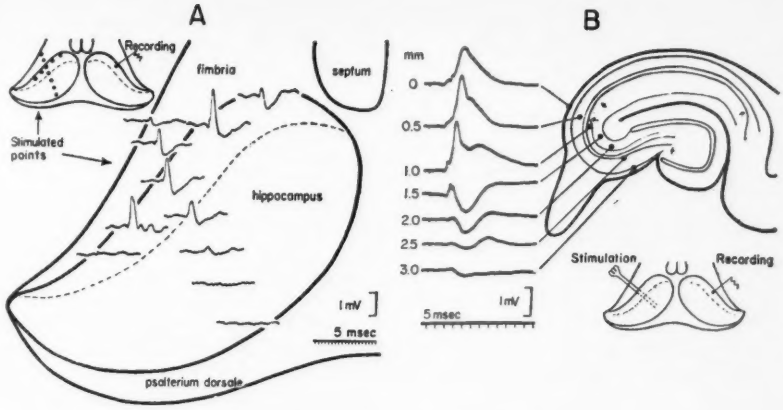

Fig. 9. Origin of interhippocampal impulses from CA3. — The recording electrode was kept fixed in CA3 of the right hippocampus while the stimulating electrode was placed at various contralateral sites. The recorded potentials are transferred to the corresponding stimulation points (see inset). — *A*. Surface stimulation of various points of the hippocampus and fimbria (rabbit). — *B*. Stimulation with an electrode penetrating the CA3 region (rabbit).

The negative wave lasted for 10—15 msec in rat, 15—25 msec in rabbit and cat. It was often composed of two portions, an early notch followed by the much greater major part (Fig. 9 B, 0 mm). On increasing the stimulus intensity the latency of the later part of the negative wave decreased more than that of the first one, and the two parts thereby fused.

The negative spike (Fig. 8 A *c*), riding on the rounded summit of the negative wave, lasted 1—3 msec in rat and 2—6 msec in rabbit and cat. The latency of this negative spike and of the small spikes on the following positive wave were decreased by augmented stimulus intensity, or by repetitive stimulation (3—30/sec).

As seen from Fig. 8 A the latency of the various components of the CA3 commissural potential differed in the three species. This is partly due to the different conduction velocities of the commissural fibres (about 7.5 m/sec in rat, 5.6 m/sec in rabbit and 3.3 m/sec in cat, ANDERSEN 1959 c) and partly to the different interelectrode distance in the three animals.

In some experiments a second negative spike occurred superimposed on the negative wave (Fig. 11 B).

Potential threshold. Comparison of the threshold of the CA3 commissural response with that of the transcallosal response gave, in one experiment, a ratio 1/20. Thus, as far as can be judged from this experiment, the threshold of the CA3 commissural potential is relatively low, even lower than that for

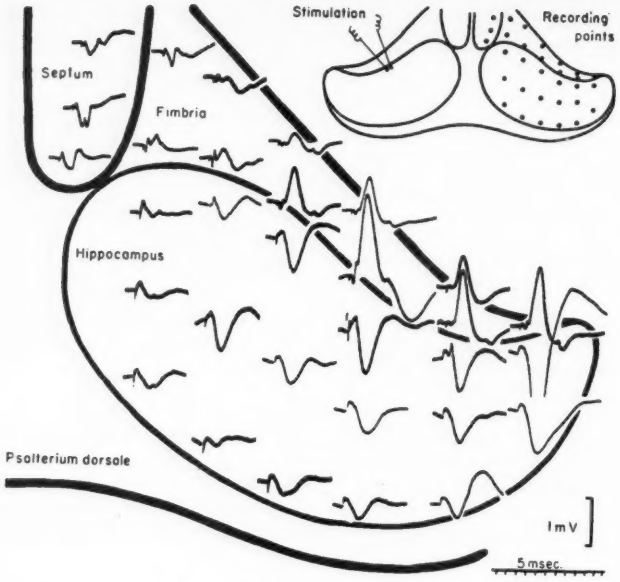

Fig. 10. Distribution of interhippocampal impulses from CA3 (rabbit). Electrode positions in inset.

the CA1 commissural potential. The latter observation was corroborated in 11 experiments with simultaneous production of the CA1 and CA3 commissural potentials.

Origin of interhippocampal impulses activating CA3. The observations reported in this section are based upon 11 experiments in 7 rabbits and 2 cats. The origin of the interhippocampal impulses to CA3, as recorded by an electrode at the fimbrio-hippocampal border, is shown in Fig. 9. The *recording* electrode was kept fixed at this point, while the *stimulating* electrode was placed at various points on the opposite fimbria and hippocampus. On surface stimulation (Fig. 9 A) the effective area occupied a small region of the hippocampus along its border to the fimbria. The contralateral point yielding the greatest responses was symmetrical to the recording electrode. No responses, or only small ones, followed stimulation of the field CA1, the subiculum, or the anterior margin of the fimbria. By separating the fimbria from the hippocampus, and placing it upon a stimulating electrode, responses could still be obtained in the contralateral field CA3, but the amplitude was greatly diminished.

When the stimulating electrode penetrated field CA3 from the alvear

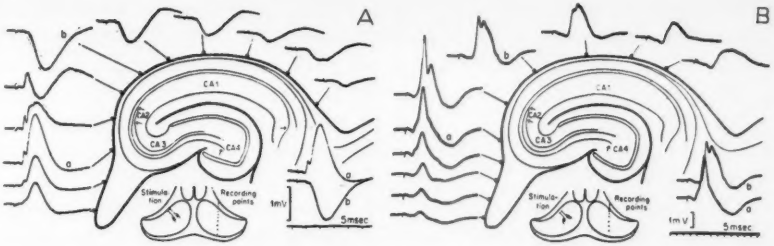

Fig. 11. Sagittal distribution of interhippocampal responses over the dorsal surfaces of the fimbria and alveus following contralateral CA3 stimulation. — *A* and *B* show two types of results. Records as demonstrated in *A* were the most common, those shown in *B* were only met with when the hippocampal cortex showed signs of high excitability. Note the phase reversal of the negative wave in the CA2 region in *A*, and in *B* the second spike and the persisting negativity in CA1. *A* from rabbit, *B* from cat.

surface (Fig. 9 B) the greatest response in the contralateral CA3 was obtained when the electrode tip was located in, or slightly superficial to, the cell body layer of its homotopic field. Small responses of reversed polarity were recorded when the electrode stimulated the inferior part of CA3 (records marked 2.5 and 3.0). Its is clearly shown that stimulation in the vicinity of the pyramidal cell layer of the superior part of CA3 is capable of producing a spike on the negative wave, whereas stimulation on the surface with the same intensity did not elicit this component. Also note that the spike latency is shorter when a point 1.0 mm below the alveus is stimulated as compared with a point 0.5 mm under the surface.

Distribution of the CA3 interhippocampal potentials. The following results are based on 12 experiments in 8 rabbits and 2 cats. Fig. 10 illustrates the distribution of the responses over the contralateral hippocampus, fimbria and septum elicited by CA3 stimulation. The stimulating electrode was kept fixed at the fimbrio-hippocampal border (see inset), and potentials were obtained from the indicated contralateral points. Both the diphasic spike and the negative wave had their greatest amplitudes in a narrow striplike region along the fimbrio-hippocampal border. In addition, the diphasic spike was recorded from the alveus for a distance of about 1—1.5 mm from the fimbria, approximately corresponding to the fields CA3 and CA2. Both the diphasic spike and the negative wave had their maximal amplitudes symmetrically to the homotopic stimulation point.

The CA3 commissural response was most readily elicited from the middle part of the longitudinal extension of CA3; the extreme rostral or temporal poles gave smaller responses.

When the recording electrode was moved across the dorsal surface of the

fimbria and the hippocampus, in a sagittal plane, the polarity of the negative wave changed approximately in the CA2 region (Fig. 11 A). In the lower right corner of Fig. 11 A records *a* and *b* are superimposed to demonstrate this polarity reversal. From CA1 a surface positive wave followed by a negative wave was recorded, much like the commissural response of this region. The amplitude of this CA1 response was diminished with increasing distance from the fimbria.

The amplitude of the early diphasic spike was greatest at the fimbrio-hippocampal border, and decreased with increasing distance from the fimbria. At approximately the border between CA2 and CA1 the spike disappeared.

Fig. 11 B illustrates the findings from a similar experiment in which the hippocampus showed signs of a relatively high excitability. At the usual recording site for the CA3 commissural response, the fimbrio-hippocampal border (record marked *a*), the potential showed one negative spike on the negative wave. As the electrode was moved across the CA1 surface a second spike was seen following the first one. The first spike showed a rapid decrease outside the CA3 region, while the second was recorded for a longer distance over the CA1 surface. In this experiment no polarity change of the negative wave took place, it remained negative along the entire field CA1. The inset in the lower right corner of Fig. 11 B shows the superimposed records from points *a* and *b*. The first spike has a longer latency in *b* compared with *a* because of the increased conduction distance. The amplitude, however, is smaller. The second spike is clearly shown in *b* but is not visible in *a*.

Following contralateral CA3 stimulation, an electrode penetrating field CA3 vertical to the pyramidal cell layer showed a gradual diminishing of diphasic spike. It disappeared at different depths in various experiments, from 0.5—1.2 mm below the surface. The negative wave showed polarity reversal at the pyramidal layer. Thus, in the layer of the apical dendrites of the CA3 pyramids the surface negative wave was always recorded as a positive wave, often of considerable amplitude. In the dentate area the recording electrode picked up a potential most often consisting of a short latency negative spike followed by a positive wave of great amplitude. Because of the complex histological structure of this region the exact interpretation of this potential is left for a future investigation. It does, however, seem likely that the early negative spike represents the activity of granule cells. More details regarding the analysis of the depth recording of CA3 are given in a following report (ANDERSEN 1959 b).

(iii) *Course of the interhippocampal impulses*

By section of various structures information was obtained about the inter-hippocampal pathways. The subsequent results are based upon 52 sections in 23 animals (7 cats, 14 rabbits and 2 rats).

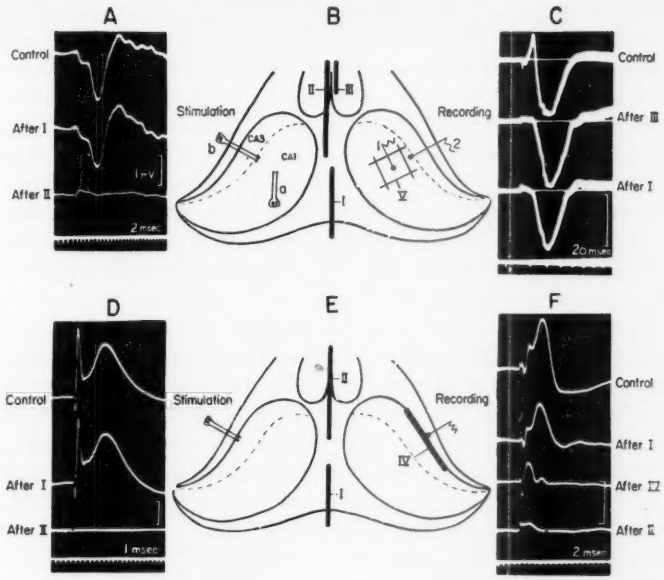

Fig. 12. Pathways of interhippocampal impulses. — *B* shows the sections referred to in *A* and *C*. —*A*. CA1 commissural potential (rabbit, *a*→1) before sectioning (control), after section of the dorsal psalterium (I), and after section of the ventral psalterium (II). — *C*. Combined CA1 and CA3 commissural response (rabbit, *b*→2) before sectioning (control), after section of the rostral part of the ventral psalterium (III) and after section of the dorsal psalterium (I). Note the independence of the CA1 commissural response in relation to the CA3 commissural response. — *E* shows the sections referred to in *D* and *F*. —*D*. The CA3 commissural response (rabbit) before sectioning (control), after section of the dorsal psalterium (I), and after subsequent section of the ventral psalterium (II). — *F*. CA3 commissural response (rabbit) before sectioning (control), after section of the dorsal psalterium (I), after a section separating the CA3 neurons from the fimbria (IV), and after a subsequent section of the ventral psalterium (II). The early diphasic spike was unaffected by section IV, whereas the negative wave was almost totally abolished. — Sections, 0.5 mm deep around the recording electrode in CA1, as indicated in *B* (V) had no influence on the CA1 commissural response.

Neither the CA1 nor the CA3 commissural responses were affected by bilateral section of both fornices rostral to the ventral psalterium, by section of the anterior commissure or by section of the dorsal psalterium. However, section of the ventral psalterium abolished both the CA1 and the CA3 commissural potentials (Fig. 12 A and D).

Two experiments deserve special comments. One is illustrated in Fig. 12 C. The stimulating electrode (B *b*) was placed 1.5 mm below the cortex, between the fields CA3 and CA1, so as to stimulate both regions. The recording electrode

(B, 2) was situated on the homotopic surface point. Before sectioning, the record consisted of a composition of the CA3 and the CA1 commissural potential. First a diphasic spike occurred, followed by a negative wave and subsequently a positive wave of long duration with a couple of small spikes superimposed. Partial section of the ventral psalterium (its rostro-ventral part) by moving the knife in the median plane in a caudal direction from the frontal pole of the brain (section III in B) abolished the CA3 commissural potential while the CA1 commissural potential with the spikes was left unchanged. Subsequent section of the dorsal psalterium (B, I) did not alter this response. Thus, the CA3 commissural fibres seem to lie rostroventrally in the ventral psalterium while the commissural fibres connecting the two fields CA1 course through the more caudo-dorsal part (*cf.* Fig. 1 B and C). Another experiment is shown in Fig. 12 F. The control record shows the usual CA3 commissural response. Section of the dorsal psalterium (E, I) caused a small decrease, apparently due to the shunting effect of the concomitant bleeding. Section IV in E which separated all the hippocampal neurons from the fimbria, abolished the negative wave almost completely, while the diphasic spike was as prominent as before. Following section of the ventral psalterium (II) also this component disappeared.

Severance of the alveus fibres by 0.5 mm deep incisions around the recording electrode (section V, in B) did not change the CA1 commissural response. The terminal commissural fibres must therefore arrive to the field CA1 through the more superficial strata of the hippocampal cortex in conformity with the findings of CAJAL (1911, vol. 2, p. 779) and LORENTE DE NÓ (1934).

Discussion

Pattern of response. The form of the commissural potentials recorded from the fields CA1 and CA3 are identical with those reported by CRAGG and HAMLYN (1957) using rabbits under nembutal anesthesia.

The CA1 commissural potential bears a striking similarity to the neocortical response to many types of synchronous afferent volleys (primary sensory response, transcallosal response) as well as to the cerebellar cortical response to most afferent stimuli, suggesting a similar activation pattern (for references see BREMER 1958). This similarity is worth mentioning for the hippocampal surface electrode is located on the white matter subjacent to the cortical grey matter, while the recording surface electrodes in the neocortex or in the cerebellar cortex are situated on the superficial, molecular, layer of the cortex. A further evaluation of this phenomenon is given in the analysis of the CA1 commissural potential (ANDERSEN 1959 a).

Subdivision of the hippocampus. The CA3 commissural response differs remarkably from its CA1 counterpart. This difference of the commissural response of CA3 (here including CA2) compared with that of CA1 may serve

as a basis for an electrophysiological subdivision of the hippocampus in two main parts. This subdivision agrees with that derived at by authors using other methods. Thus, based upon observations of the terminal degeneration of interhemispherical hippocampal fibres in the rat BLACKSTAD (1956) divided the hippocampus into two main regions, *i. e.* between the fields CA2 and CA1. This line of division is the same as CAJAL (1911) used in his cytoarchitecture studies to separate the hippocampus into regio superior and regio inferior. Embryologically these two regions develop from different cell groups (TILNEY 1938). Recently, FLEISCHHAUER and HORSTMANN (1957) have also shown a histochemical differentiation between these two regions. Intravenous injection of a dye, dithizon, in a variety of animals gave distinct uptake of the dye in the cell body layers of the dentate area and of the CA3, but none in the CA1 (respectively h_3 and h_2 of ROSE (1926)). Finally, COGGESHALL and MACLEAN (1958) have shown that the CA3 pyramidal cells are destroyed after injection of the drug 3-acetylpyridine, while the CA1 cells seem unaffected.

Origin of interhippocampal impulses. The low stimulus thresholds observed when the stimulating electrodes were placed in the cell body layer, or among the apical dendritic shafts, indicate that these structures are the main sources of the commissural volley from the hippocampus. This applies to CA1 as well as to CA3. Although a variety of cell types have been described in the mentioned layers (CAJAL 1911, LORENTE DE NÓ 1934) it seems reasonable to ascribe most of the activation to the pyramidal cells, since they are the greatest and most numerous of the nerve cells present.

The effect of stimulation in the region of the apical dendritic shafts might be explained by spread of current to the pyramidal layer. However, with the electrodes and the stimulus parameters employed, the excitable area seemed to be fairly small. A displacement of 0.2 mm (sometimes as little as 50 μ) of the stimulating electrode along the direction of the apical dendritic shafts could result in a threshold fall of about 80 per cent, or in the abrupt onset of a well developed commissural potential when constant stimulus intensity was used (Fig. 4 A, 1.0—1.2 mm; Fig. 4 C, 1.2—1.4 mm and 3.2—3.4 mm). The error of electrode location was less than 100 μ. Further, when the stimulating electrode was placed at a certain distance from the pyramidal layer, the stimulus threshold was much lower on the side of the apical dendrites than on the other side (Fig. 4), pointing against the current spread as the only explanatory factor of the observed results. This suggests that the membrane of the proximal parts of the CA1 apical dendrites is electrically excitable (see also ANDERSEN 1959 a). This suggestion is contrary to the interpretation given by PURPURA and GRUNDFEST (1956) concerning the properties of the neocortical dendritic membranes. However, their assumption rests upon indirect evidence as the authors have to propose a specific blocking action of *d*-tubocurarine upon the synapses of the investigated apical dendrites.

This assumption is complicated further by the report of the same authors of the reversal of the effect of *d*-tubocurarine upon the local cortical potential when the animals were heparinized. Further, CHANG (1953 b) found that topical application of *d*-tubocurarine did not affect the local cortical potential. Taking the histological complexity of the neocortex into account, it is not unlikely that the theory of PURPURA and GRUNDFEST (1956) concerning the electrical inexcitability of the entire dendritic membrane may have to be somewhat modified.

Termination of interhippocampal impulses. The localization value of the CA1 and the CA3 commissural responses is naturally dependent upon whether they are produced by direct or by relayed impulses from the contralateral fields in question.

Concerning the interpretation of the interhippocampal responses the present author has arrived at some conclusions which differ from those of CRAGG and HAMLYN (1957). These authors did not consider the CA1 potential as a true commissural one, but interpreted it as due to CA1 neurons responding to impulses from ipsilateral CA4 or dentate granule cells which in turn were primarily activated by the commissural volley.

On the basis of experiments with threshold measurement, depth recording, paired shocks, repetitive stimulation, anoxia, local application of strychnine and nembutal, and section of various fibre connections (for a discussion of these problems see ANDERSEN 1959 a, b), the following conclusions were drawn. The surface negative wave of the CA3 commissural response and the deep negative wave of the CA1 commissural response are considered as summated excitatory postsynaptic potentials (EPSP) produced in the basal and apical dendrites, respectively, of the pyramidal cells. The greater part of both EPSPs seems to be produced by direct commissural impulses.

The assumption of a direct activation of CA1 neurons by commissural impulses is in agreement with the findings of BLACKSTAD (1956) concerning the terminal degeneration of interhemispherical fibres in the regions discussed. However, the interpretation presented above differs from the conclusion drawn by CRAGG and HAMLYN (1957) as stated above. They concluded that the marked facilitation of the response evoked in CA1 by contralateral repetitive stimulation of 8/sec pointed to the involvement of more than one synapse. This synaptic relay was believed to take place in the dentate area. However, the facilitation by repetitive stimulation does not seem sufficient to exclude the contribution of direct impulses in the production of the reported potentials (ANDERSEN 1959 a). Further, if the interpretation of CRAGG and HAMLYN (1957) is accepted, the interhippocampal impulses to CA1 must traverse three synapses (Fig. 1 A), the first in the dentate area, the second between the mossy fibres of the granule cells and the CA3 pyramids, and the third between the Schaffer collaterals of the CA3 axons and the apical dendrites of the CA1 neurons. The behaviour of the CA1 commissural response in conditions of fatigue, and anoxia, as well as its excitability cycle (ANDERSEN 1959 a), strongly

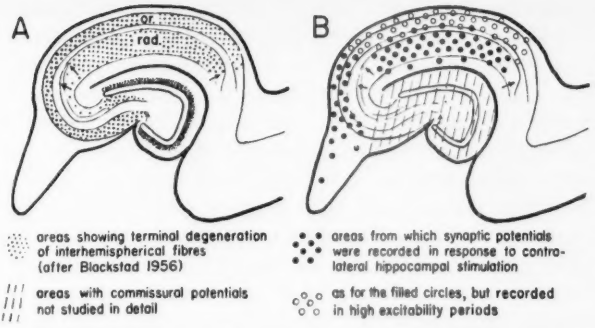
A
or.
rad.
B
areas showing terminal degeneration of interhemispherical fibres (after Blackstad 1956)
areas with commissural potentials not studied in detail
areas from which synaptic potentials were recorded in response to contralateral hippocampal stimulation
as for the filled circles, but recorded in high excitability periods

Fig. 13. Diagram illustrating the results regarding termination of interhippocampal fibres (*A*) as revealed by experimental histological methods (BLACKSTAD 1956) and (*B*) by electrophysiological methods in the present study.

indicates that the major part of this potential is not evoked through a three-synapse pathway, but through only one synapse. This is presumably situated between the commissural terminal knobs and the CA1 neurons.

Finally CRAGG and HAMLYN (1957) used rabbits under barbiturate anesthesia which markedly depresses postsynaptic summation and increases the total cortical unresponsive period following single shock activation (HEINBECKER and BARTLEY 1940, JARCHO 1949). This fact would similarly favour the assumption that a part of the evoked CA1 responses observed by CRAGG and HAMLYN were due to directly activated CA1 neurons by the commissural volley.

Under normal conditions the potentials directly evoked in CA1 by contralateral CA3 stimulation did not differ from the usual CA1 commissural response. However, in periods of moderately increased excitability, the surface negative wave started earlier and was of greater amplitude, and at times the response consisted only of a pure negative wave (Fig. 5). This is interpreted as an activation of parts of the CA1 neurons lying near the alvear surface, presumably the basal dendrites and/or the cell bodies. The pure negative wave is considered as a summated EPSP elicited by synaptic bombardment of the structures mentioned above (ANDERSEN 1959 a).

The findings and interpretation concerning the distribution of the summated EPSPs in the hippocampal cortex in response to stimulation of symmetrical and non-symmetrical parts of the contralateral hippocampus are in agreement with the observations of BLACKSTAD (1956). BLACKSTAD observed terminal degeneration of interhemispherical fibres in the stratum oriens (Fig. 13, or.) of CA3, CA2 and CA1. The stratum radiatum (rad.) showed heavy degeneration only in CA1 and in a small dentate-near part of CA3. In the fields CA2 and the major (CA2-near) part of CA3 this layer showed very scattered degeneration. Fig. 13 B demonstrates that the negative waves, interpreted as

EPSPs activated directly by a commissural volley, were recorded in those regions of the hippocampus where BLACKSTAD (1956) observed terminal degeneration following destruction of the contralateral hippocampus and dentate area. The physiological findings made in this study suggest that the activation of the CA3 neurons by a commissural volley is effected by a direct synaptic bombardment of their basal dendrites, an assumption which is in agreement with the anatomical results of BLACKSTAD (1956).

In the field CA1 the commissural activation of the apical dendrites seems to be the most effective one because the EPSP produced by a commissural volley are normally recorded from the stratum radiatum only (filled circles in Fig. 13 *B*). However, in periods of higher excitability, contralateral CA3 stimulation could produce a surface negative wave, presumed to be a summated EPSP taking its origin in the basal dendrites and/or the cell bodies of the CA1 pyramids as well (open circles of Fig. 13 B). A reasonable explanation would be that the terminal interhippocampal fibres in the stratum oriens of CA3, as well as of CA1, mainly take their origin from the contralateral CA3, and that the terminal commissural fibres to the stratum radiatum of CA1 originate from the contralateral CA1 neurons only. These assumptions require verification by further anatomical experiments with small, localized hippocampal lesions.

The point-to-point[1] localization between homotopic, circumscribed small areas within the fields CA1 or within the fields CA3 of the two sides is comparable to the similar connection between symmetrical areas in the neocortex by way of the corpus callosum (CURTIS and BARD 1939, CURTIS 1940, MCCULLOCH and GAROL 1941, CHANG 1953 a). The direct activation of CA1 neurons from areas of the contralateral hippocampus outside the symmetrical point, *i. e.* from the contralateral CA3 bears some resemblance to the observations of MCCULLOCH and GAROL (1941) and of MCCULLOCH (1944). These authors found that strychninization of some areas fired only the homotopic areas while other areas fired contralateral heterotopic areas as well. The anatomical basis for such observations had been found earlier by METTLER (1936).

Termination of interhemispherical impulses in the dentate area. Within the dentate area the histological picture is more complex than in CA3 and CA1. For this reason the interpretation of the potentials recorded from this area in response to contralateral symmetrical stimulation offers greater difficulties. Therefore, on the basis of the present investigation no final conclusions can be drawn concerning the exact termination of the interhemispherical impulses to this region. Some observations related to this problem will be discussed elsewhere (ANDERSEN 1959 b).

Generally, the interhemispherical potentials recorded from the dentate area seem to indicate a synaptic relation of high effectivity and short latency within

[1] It is admitted that the term point-to-point is not quite correct as the commissural volley activates an area rather than a point. Nevertheless, the expression is used because it refers to the point where *maximal* responses were obtained in response to contralateral stimulation.

the granule cell layer. This finding is in agreement with similar observations of CRAGG and HAMLYN (1957). Records indicating short latency dentate cell activation have also been obtained by GREEN and ADEY (1956) and by VON EULER, GREEN and RICCI (1958) following stimulation of the dorsal fornix. The assumption of the activation of the granule cells by contralateral CA3 or dentate stimulation is in agreement with the histological observations of BLACKSTAD (1956). Even if the physiological observations are compatible with such an interpretation, they can — because of the histological complexity in this area — not give any direct and positive evidence as to whether the volley from the contralateral CA3 or dentate area activates the granule cells only, or the giant pyramids in the hilus of the dentate fascia only, or if it activates both of these structures, or even in addition other cell types in this region.

Pathways of the interhippocampal impulses. The findings concerning the interhippocampal pathways reported in this paper are in agreement with the normal anatomical observations (CAJAL 1911, LORENTE DE NÓ 1934) and with the experimental results of BLACKSTAD (1956) and of CRAGG and HAMLYN (1957). The CA3 and the CA1 commissural responses are not affected by section of the dorsal psalterium, whereas section of the ventral psalterium abolishes them. The fornix and the anterior commissure do not seem to mediate direct impulses between the two hippocampi.

The indication, suggested by some of the observations reported (Fig. 12), that the commissural fibres, interconnecting the CA1 and CA3 fields of the two sides, respectively, occupy different areas within the ventral psalterium requires further investigation. However, the assumption does not seem unlikely when comparison is made with the topographical arrangement of the fibres in the corpus callosum (CHANG 1953 a).

Summary

1. The hippocampus and the fimbria have been stimulated electrically and evoked potentials have been recorded from the same structures contralaterally in cat, rabbit and rat. Both surface and depth stimulation and recording have been employed.

2. Two types of commissural responses could be distinguished, one between the fields CA1, and the other between the fields CA3.

3. The *CA1 commissural response* (Fig. 2) consisted of a surface positive/negative diphasic wave of 10—20/30—100 msec duration. In periods of high excitability, or following stronger or repetitive stimulation, one or several spikes of 3—5 msec duration, usually negative, were superimposed upon the positive wave.

4. The *CA3 commissural response* (Fig. 8) consisted of an early diphasic spike

of 1—4 msec duration followed by a negative wave of 10—25 msec duration and a late positive wave. Following stronger stimulation a negative spike was superimposed upon the negative wave as well as upon the late positive wave.

5. The stimulus threshold for the CA1 and the CA3 commissural potentials were both lower than that for the transcallosal potential.

6. The interhippocampal impulses recorded from points in CA1 or in CA3 were elicited by stimulation of the homotopic points. In both CA1 and CA3 depth stimulation revealed maximal contralateral responses when the stimulating electrode tip was situated in the layers of the pyramidal cell bodies and their apical dendritic shafts (Fig. 4 and 9 B).

7. Interhippocampal impulses *from* CA1 activated a circumscribed area symmetrical to the stimulating electrode with a maximum at the homotopic point (Fig. 6). The commissural volley arrived at the stratum radiatum of CA1 where it presumably activated the apical dendritic shafts (Fig. 7), eliciting a negative wave (EPSP), eventually with one or two negative spikes superimposed.

8. CA1 neurons could also be activated from the contralateral CA3 field, either directly or indirectly. The direct activation took place on alveus-near parts of the CA1 neurons.

9. Interhippocampal impulses *from* CA3 normally were recorded from an elongated area within the contralateral CA3, with maximal responses at the homotopic point (Fig. 10). Depth recording indicated that the activation took place by eliciting a negative wave (EPSP) in the layer of the basal dendrites.

10. There is some evidence that stimulation of the dentate area or of CA3 evokes a short latency spike from the granule cells of the contralateral dentate area, but the possibility that the responses obtained are due to activity of CA4 neurons can not be excluded.

11. The CA1 as well as the CA3 interhippocampal impulses are mediated by fibres in the ventral psalterium (ventral hippocampal commissure). Section of the dorsal psalterium, the anterior commissure or both fornices left the two commissural responses intact, whereas both were abolished by ventral psalterium section.

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Reduction of CO_2 Stores of Man due to Muscular Exercise

By

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Received 3 March 1959

Abstract

Andersen, K. L. and B. Bugge-Asperheim. Reduction of CO_2 stores of man due to muscular exercise. *Acta physiol.* 1959. *47.* 91—96. — The respiratory recovery from exercise has been studied using a spirometer system of the closed circuit type which gives a continuous recording of the gas exchange.

It is demonstrated that bicycling 1,500 kgm. in 1 minute leads to an increased lactic acid content of circulating blood which is followed by an excess blowing-off of CO_2 in the first minutes of recovery, amounting to 2—3 % of the estimated total CO_2 stores of the body.

None or only a slight tendency to CO_2-retention was observed during a two hours post-exercise period, in which the gas exchange was recorded continuously.

It has been shown that heavy muscular exercise is followed by excess CO_2-output beyond that produced by the metabolic processes. This excess CO_2-output is caused by anaerobic acid products in the circulating blood (Hill, Long and Lupton 1924, Margaria, Edwards and Dill 1933).

Low carbon dioxide output relative to oxygen intake has been observed during the recovery period, resulting in abnormally low respiratory quotient (R. Q.). This has been demonstrated as late as 60 min. after cessation of work.

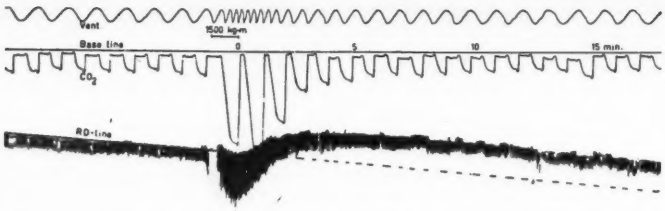

Fig. 1. The spirometer tracings show from above: Pulmonary ventilation, base line, CO_2-output in 1 minute periods, spirometer curve with respiratory difference line (R. D. line) drawn through the expiratory ends of the spirometer tracings.

These observations show that a CO_2 retention takes place during the recovery period, compensating the excess CO_2-output occurring during and shortly after exercise.

Little evidence is available to show how long it takes before the CO_2-content of the body has reached the original level. Nor do we have any information as to the extent of CO_2-reduction necessary before the organism reacts with a CO_2-retention.

These problems have been studied by measuring the gas exchange continuously before and after exercise by means of a closed spirometer system, by which changes in the total contents of respiratory gases can be read from the recorded spirometer curve.

Methods and experimental procedures

The spirometer used was that originally constructed by SCHOLANDER (1940) for zoophysiological work, later improved and dimensioned for human physiological work by ERIKSON, SCHOLANDER and IRVING (1951).

A bicycle ergometer has been used as a working machine, the subject sitting in a comfortable chair instead of on an ordinary bicycle seat. This arrangement has several advantages and allows studying the subject during work and recovery without disconnecting from the apparatus and without moving the subject. In addition only small variations in the gas filling level of the lungs take place which is important when the changes of the gas content of the total body are to be calculated from the spirometer tracings.

Blood lactate was determined from the capillary blood by using STRÖM's modification of Barker and Summerson's colorimetric method (1949).

Results

Work loads of 1,500 kgm performed in 1 minute were used. Fig. 1 shows section of a spirometer tracing. The exercise performed leads to an elevation of the R. D. line of the spirometer curve.

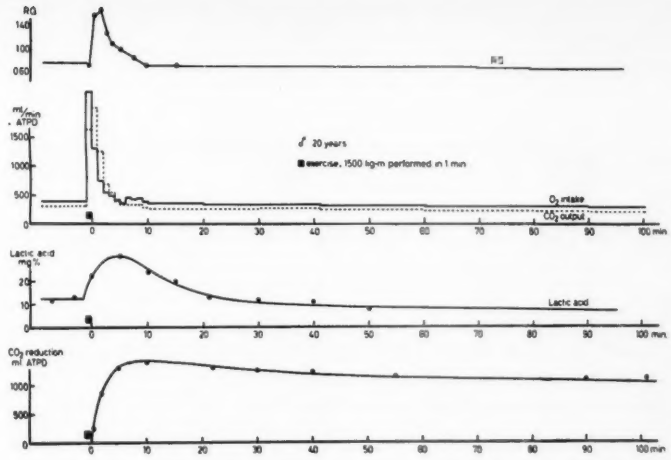

Fig. 2. O_2-intake and CO_2-output measured from the spirometer tracings shown in Fig. 1. The figure shows further R. Q., blood lactate, and the reduction of the CO_2 stores of the body.

From the tracings the O_2-intake and CO_2-output have been measured. These data are shown in Fig. 2, including the calculated R. Q. and the corresponding values of blood lactate.

After resting values of O_2-intake and CO_2-output have been reached, the volume of the gas in the lungs and the respiratory tract is practically the same

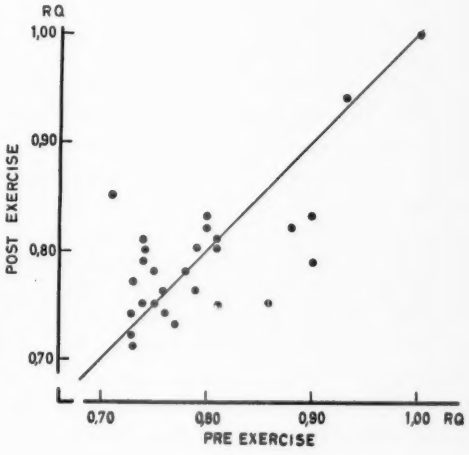

Fig. 3. Resting values for pre- and postexercise R. Q. in 28 experiments where the subjects performed 1,500 kgm in one minute. The postexercise value is calculated as the average of a 10 min period, mostly taken between the 15th and 25th minute after cessation of work and after the gas exchange had reached resting level. The diagonal line shows equal values.

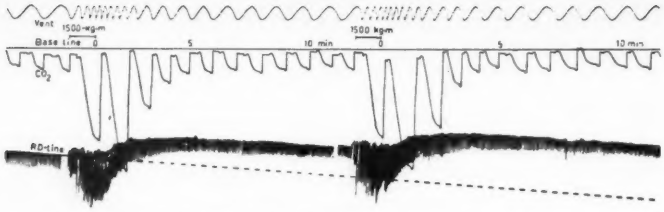

Fig. 4. Spirometer tracings. See Fig. 1. Two work loads of 1,500 kgm performed.

as before exercise, because the subject is sitting in the same position. Consequently the elevation of the R. D. line above the pre-exercise level is mainly due to a reduction of the CO_2-stores of the body.

In repeated experiments the subject has been studied continuously for as long as 2 hours after the work, and the results revealed none or only a small retention of CO_2.

In Fig. 3 are shown pre- and postexercise resting values for R. Q. The postexercise R. Q. is calculated as the average of a 10 min period after stable resting values for O_2-intake and CO_2-output have been established. As seen there is some variability in the data, however, no abnormal R. Q. values were recorded in the postexercise period, which shows that no CO_2-retention took place in any of these experiments.

In another series of experiments the subject bicycled 1,500 kgm two periods of 1 minute length, with an interval of 11 min.

Fig. 4 shows section of spirometer tracing values from such an experiment. After the second work period a considerable elevation of the R. D. line above the original was found. During the recovery period which was studied up to 90 min. after cessation of exercise, the elevated R. D. line parallels the pre-exercise R. D. line, showing that no CO_2 retention takes place.

Ten min after the cessation of the last work load the reduction of the CO_2 stores of the body was measured to be 2,700 ml.

Discussion

ERIKSON (1957) studied gas exchange of subjects bicycling 1,125 kgm using the same method as in this study, and could not find any CO_2-retention during a 40—60 min recovery period. The same author studied gas exchange after 70 yards run, and after this type of exercise subresting values of CO_2 during recovery were recorded, showing that this excessive load resulted in a CO_2-retention.

In relation to other gases in the body the total amount of CO_2 is considerable. It has been calculated that a subject weighing 70 kg contains about 1.0 litre

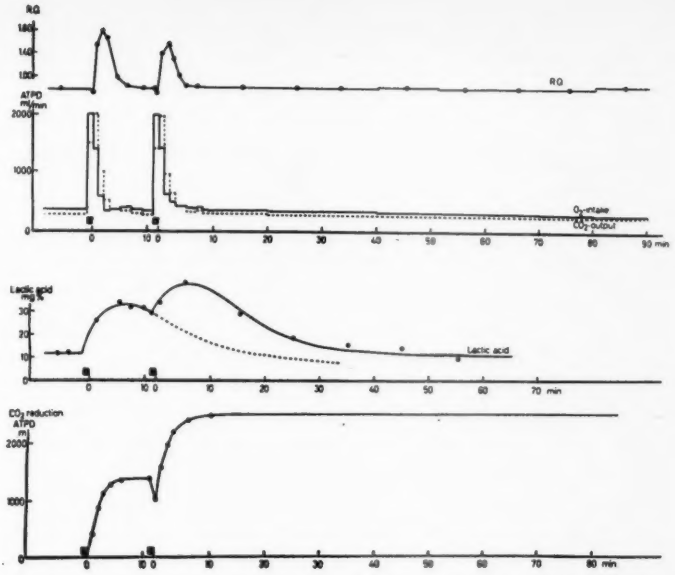

Fig. 5. O_2-intake and CO_2-output measured from the spirometer tracings shown in Fig. 3.

of O_2 and N_2. The human body may give away approximately 120 l of CO_2 (FARHI and RAHN 1955).

Experiments on animals have shown that quantitative changes of the CO_2 stores within the body may take place. FREEMAN and FENN (1953) observed that the total amount of CO_2 decreased in rats which lived in a low oxygen atmosphere and increased in an atmosphere rich of CO_2. Retention of CO_2 is also found in man inspiring air with a high carbon dioxide content (HERXHEIMER and KOST 1932). IRVING, FERGUSON and PLEWES (1930) showed that in eviscerated cats the pulmonary ventilation led to such a considerable excess CO_2-output, that some of it must originate from the CO_2 chemically bound to the bones. This shows that the CO_2 of the bones can be mobilized and excreted through the lungs.

The reduction of CO_2 stores found in this study amounts to 2—3 % of the estimated total CO_2 content of the body. The corresponding reduction of pCO_2 of the arterial blood is believed to be too small to cause an immediate hypoventilation and a corresponding CO_2-retention.

Although no CO_2-retention could be observed during a 2 hours period following the work, it may be that the original CO_2-level within the body is re-established during sleep and rest the following night.

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